DOPAMINE TRANSPORTER INHIBITORS AND THEIR USE

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to compounds designed to inhibit dopamine transporter protein, therapeutic uses of such compounds, and a method of rational design of such compounds.

Background of the Invention

Dopamine is a neurotransmitter and plays an essential role in normal brain functions. Abnormal dopamine signaling in the brain has been implicated in many pathological conditions, including drug (cocaine) abuse, depression, anxiety, eating disorders, alcoholism, chronic pain, obsessive compulsive disorders and Parkinson's Disease.

In the brain, dopamine is synthesized in the pre-synaptic neurons and released into the space between the pre-synaptic and post-synaptic neurons. Dopamine molecules then bind to and activate the dopamine receptors on the postsynaptic neurons. Dopamine molecules are then transported through the dopamine transporter protein (DAT) back into the pre-synaptic neurons, where they are metabolized. In conditions such as cocaine abuse, cocaine binds to the dopamine transporter and blocks the normal flow of dopamine molecules. Excess concentrations of dopamine cause over-activation of dopamine receptors. In other conditions such as Parkinson's Disease, lack of sufficient dopamine receptors in the brain causes insufficient activation of dopamine receptors. Thus, modulation of dopamine signaling constitutes an attractive approach for therapeutic intervention for conditions in which normal dopamine signaling is disrupted.

Cocaine abuse is one of the greatest concerns of the American public today, and has therefore become a focus of medical, social, and political debate. Cocaine is one of the most addictive substances known, and cocaine addicts may lose their ability to function at work or in interpersonal situations. Although cocaine potently inhibits the reuptake of both norepinephrine (NE) and serotonin (5-HT), many lines of evidence indicate that its ability to act as a reinforcer stems from its ability to inhibit the reuptake of dopamine (DA) into dopaminergic neurons. Cocaine exerts this effect via specific interaction with DA transporter (DAT) proteins (cocaine receptor) located

on DA nerve terminals. This increase of dopaminergic transmission in the reward mediating brain mesolimbic system is the essence of the dopamine hypothesis for cocaine action.

In the pursuit of discovery of a cocaine antagonist as potential therapy for the treatment of cocaine abuse, substantial efforts have been focused on modifications of cocaine itself; this has led to a wealth of information about the structure-activity relationships (SARs) of cocaine analogs. Despite intense research efforts in this area, very few compounds with significant cocaine antagonist activity have been reported. Therefore, there remains a need for lead compounds capable of antagonizing all or some of cocaine action.

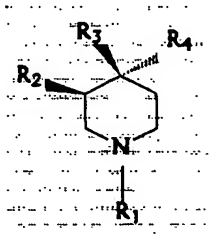
Therefore, there remains a need for compounds that can modulate dopamine signaling through the binding to the DAT. Such compounds will then be implemented therapeutic protocols in the treatment of conditions in which dopamine signaling plays a role.

SUMMARY AND OBJECTS OF THE INVENTION

It is an object of the invention to provide compounds which modulate abnormal dopamine signaling in the brain.

It is another object of the invention to provide compounds which are antagonistic of cocaine.

In one aspect, the invention provides a compound of formula (I):



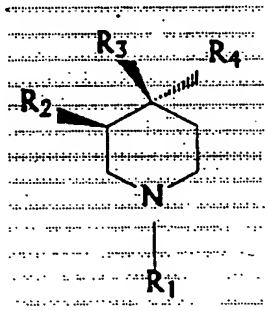
(I)

wherein R_1 is a hydrogen; linear (C_1-C_7) alkyl; branched or cyclic (C_3-C_7) alkyl; halogenated linear, branched or cyclic alkyl; aryl or alkylaryl, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aryl or alkylaryl or connected to the aryl or alkylaryl by a C_1-C_5 alkyl; or an aromatic ring containing one or more hetero atoms selected from N, S, and O, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear

alkyl, nitro, alkoxyl, and hydroxyl; R_2 and R_4 are, independently, linear (C_1-C_7) alkyl; branched or cyclic (C_3-C_7) alkyl; halogenated linear, branched or cyclic alkyl; aryl or alkylaryl, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aryl or alkylaryl or connected to the aryl or alkylaryl by a C_1-C_5 alkyl; an aromatic ring containing one or more hetero atoms selected from N, S, and O, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl and an amino group directly linked to the aromatic ring or connected to the aromatic ring by a C_1-C_5 alkyl; C(O)-R', wherein R' is linear (C_1-C_7) alkyl, branched or cyclic (C_3-C_7) alkyl, halogenated linear, branched or cyclic alkyl, aryl or alkylaryl, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aryl or alkylaryl or connected to the aryl or alkylaryl by a C_1-C_5 alkyl, or an aromatic ring containing one or more hetero atoms selected from N, S, and O, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aromatic ring or connected to the aromatic ring by a C_1-C_5 alkyl; primary, secondary or tertiary (C_1-C_7)alcohol; C(O)OR" wherein R" is a linear (C_1-C_7) alkyl, branched or cyclic (C_3-C_7) alkyl, halogenated linear, branched or cyclic alkyl, aryl or alkylaryl, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aryl or alkylaryl or connected to the aryl or alkylaryl by a C_1-C_5 alkyl, or an aromatic ring containing one or more hetero atoms selected from N, S, and O, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aromatic ring or connected to the aromatic ring by a C_1-C_5 alkyl; C(O)OR"' wherein R"' is a hydrogen, linear (C_1-C_7) alkyl, branched or cyclic (C_3-C_7) alkyl, halogenated linear, branched or cyclic alkyl, aryl or alkylaryl, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aryl or alkylaryl or connected to the aryl or alkylaryl by a C_1-C_5 alkyl, or an aromatic ring containing one or more hetero atoms selected from N, S, and O, optionally substituted with one or more substituents selected from the group consisting of F, Cl,

Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aromatic ring or connected to the aromatic ring by a C_1-C_5 alkyl; C(O)NH-R"" or NHC(O)-R"" wherein R"" is a hydrogen, linear (C_1-C_7) alkyl, branched or cyclic (C_3-C_7) alkyl, halogenated linear, branched or cyclic alkyl, aryl or alkylaryl, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aryl or alkylaryl or connected to the aryl or alkylaryl by a C_1-C_5 alkyl, or an aromatic ring containing one or more hetero atoms selected from N, S, and O, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aromatic ring or connected to the aromatic ring by a C_1-C_5 alkyl; and R_3 is F, Cl, Br, I, OH, OR"""" or OC=OR"""", wherein R"""" is an alkyl, aryl, aromatic ring containing one or more hetero atoms, or R_3 is a covalent bond replacing the hydrogen in a hydroxyl group of R_2 when R_2 is alcohol or hydroxyl.

In another aspect, the invention provides a method of inhibiting cocaine action in a subject in need of such inhibition comprising administering to the subject an effective amount of a compound of formula (I):



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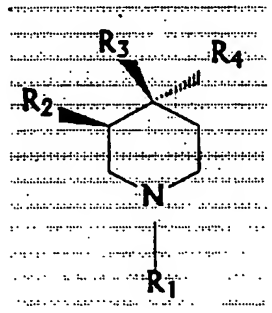
NHC(O)-R"" wherein R"" is a hydrogen, linear (C_1-C_7) alkyl, branched or cyclic (C_3-C_7) alkyl, halogenated linear, branched or cyclic alkyl, aryl or alkylaryl, optionally substituted with one or more substituents selected -from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aryl or alkylaryl or connected to the aryl or alkylaryl by a C_1-C_5 alkyl, or an aromatic ring containing one or more hetero atoms selected from N, S, and O, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aromatic ring or connected to the aromatic ring by a C_1-C_5 alkyl; and R_3 is F, C_1, Br, **1**, OH, OR"" or OC=OR"", wherein R"" is an alkyl, aryl, aromatic ring containing one or more hetero atoms, or R_3 is a covalent bond replacing the hydrogen in a hydroxyl group of R_2 when R_2 is alcohol or hydroxyl.

In yet another aspect, the invention provides a method of control of dopamine flow in a subject in need of such control comprising administering to said subject an effective amount of a compound of formula (I):



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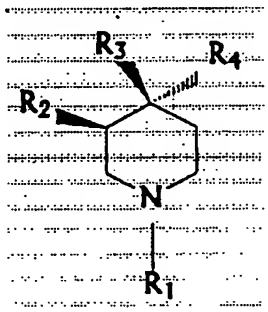
In a further aspect, the invention provides a method of modulating dopamine reuptake action in a subject in need of such action comprising administering to said subject an effective amount of a compound of formula (I):



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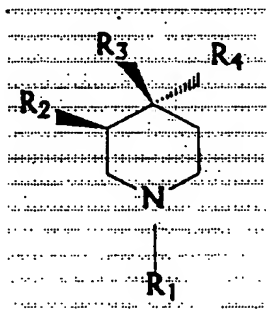

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wherein R, is methyl, ethyl, propyl;

R_2 is -C(O)-R" or -CHOH-R"wherein R" is 4-methyl-phenyl, 4-ethyl-phenyl, 3,4 dimethyl-phenyl, 3,4-difluoro-phenyl, 2-chloro-phenyl, 3-chloro-phenyl, 4-chlorophenyl, 2,4-dichlorophenyl, 3,4-dichloro-phenyl, 4-bromo-phenyl or 4-iodo-phenyl;

R_3 is hydroxy; and

R_4 is 4 methyl-phenyl, 4-ethyl-phenyl, 3,4-dimethyl-phenyl, 3,4-difluoro-phenyl, 2-chloro-phenyl, 3-chloro-phenyl, 4-chloro-phenyl, 2,4-dichloro-phenyl; with the proviso that R' cannot be 4-methyl-phenyl when R_1 is methyl and R_4 is 4-methyl-phenyl.

In yet another aspect, the invention provides a compound selected from the group consisting of 3,4-dichlorophenyl 4-(3,4-dichlorophenyl)-4-hydroxy-1-methyl-3-piperidyl ketone; 2,4-dichlorophenyl 4-(2,4-dichlorophenyl)-4-hydroxy-1-methyl-3-piperidyl ketone; 3- [Hydroxy(4-methylphenyl)methyl] –1 -methyl-4-(4-methylphenyl)piperidin-4-ol; 4-(3,4-dichlorophenyl)-3-[(3,4-dichlorophenyl)hydroxymethyl)]-1-methylpiperidin-4-ol; 4-(2,4-dichlorophenyl)-3-[(2,4-dichlorophenyl)hydroxymethyl] -1-methylpiperidin-4-ol; 8-aza-1, 5bis(4-

methylphenyl) δ-methyl-2,4-dioxabicyclo[4,4,0]decan-3-one; and 4-chloro-3-methylacetophenone; 4-chloro-3-methyl phenacylchloride; 4-chlorophenyl 4-(4-chlorophenyl)-4-hydroxy-1-methyl-3-piperidyl ketone; 4-bromophenyl 4-(4-bromophenyl)-4-hydroxy-1-methyl-3-piperidyl ketone; 4-hydroxy-4-(4-iodophenyl)-1-methyl-3-piperidyl 4-iodophenyl ketone; 4-ethylphenyl 4-(4-ethylphenyl)-4-hydroxy-l-methyl-3-piperidyl ketone; 2-chlorophenyl 4-(2-chlorophenyl)-4-hydroxy-l-methyl-3-piperidyl ketone; 3-chlorophenyl 4-(3-chlorophenyl)-4-hydroxy-l-methyl-3-piperidyl ketone; 3,4-difluorophenyl 4-(3,4-difluorophenyl)-4-hydroxy-l-methyl-3-piperidyl ketone; 3,4-dimethyl 4-(3,4-dimethylphenyl)-4-hydroxy-l-methyl-3-piperidyl ketone; 4-chloro-3-methylphenyl 4-(4-chloro-3-methylphenyl)-4-hydroxy-l-methyl-3-piperidyl ketone; 1-ethyl-4-hydroxy-4-(4-methylphenyl)-3-piperidyl 4-methylphenyl ketone; 4-chlorophenyl 4-(4-chlorophenyl)-1-ethyl-4-hydroxy-3-piperidyl ketone; 3,4-dichlorophenyl 4-(3,4-dichlorophenyl)-1-ethyl-4-hydroxy-3-piperidyl ketone; 3,4-dichlorophenyl 4-(3,4-dichlorophenyl)-4-hydroxy-1-(2-phenylethyl)-3-piperidyl ketone; 4-bromophenyl 4-(4-bromophenyl)-4-hydroxy-l-(2-phenylethyl)-3-piperidyl ketone;

3,4-dichlorophenyl 4-(3,4-dichlorophenyl).-4-hydroxy-l-(3-phenylpropyl)-3-piperidyl ketone; and 4-bromophenyl 4-(4-bromophenyl)-4-hydroxy-l-(3-phenylpropyl)-3-piperidyl ketone.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram of the chemical structures of R-cocaine and cocaine analog WIN-35065.

Figure 2 is a schematic diagram of a pharmacophore according to the invention.

Figure 3 depicts the chemical structure of lead compound **3**.

Figure 4 is a graph of inhibition of dopamine uptake by cocaine alone or by cocaine in the presence of compound **3**. The graph shows the effect of cocaine on [^{3}H]-dopamine uptake in the presence of lead compound **3**. (solid circles, cocaine alone; open squares, 50 nM of **3** + cocaine; solid triangles, 200 nM of **3** + cocaine; open circles, 500 nM of **3** + cocaine).

Figure 5 shows one schematic diagram for the synthesis of analogs **3-11**.

Figure 6 shows two schematic diagrams for the synthesis of analogs **12-27** of compound **3**.

Figures 7 (A-D) are superpositions of low energy conformations of lead compound **3** and analog **6**, analog **7**, analog **9**, and analog **11**.

Figures 8 (A-B) are superpositions of low energy conformations of analogs lead compound **3** and analogs **12** and **17**, and analog **16**, respectively.

Figure 9 (A-B) is a graph illustrating the effect of cocaine alone or analog **6** alone on locomotor activity in mice. The graph shows behavioral effects of cocaine (circles) and compound **6** (squares). Both compound **6** ($F_{5,61} = 11.29$, $P < 0.001$) and cocaine ($F_{5,74} = 30.5$, $P < 0.001$) produced significant and dose-dependent increases in the distance traveled (A) in male Swiss-Webster mice. Similarly, compound **6** ($F_{5,61} = 6.89$, $P < 0.001$) and cocaine ($F_{5,74} = 8.31$, $P < 0.001$) significantly increased stereotypic movements (B). The distance traveled and the stereotypic movement responses in saline control group were 3517 ± 325.6 cm and 1299 ± 71, respectively. The corresponding numbers for 10% DMSO vehicle control group were 3402 ± 363 cm and 1159 ± 80. *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ as compared to the responses in the corresponding vehicle control group by Tukey's post hoc test.

Figure 9 (C-D) is a graph depicting the results of the discriminative stimulus testing in rats of cocaine alone or analog **6** alone. The graph shows behavioral effects of cocaine (circles) and compound **6** (squares). The results of the discriminative stimulus testing in rats(n = 5 to 7) are shown in panels C and D. The data points in the figure represent the mean ± S.E.M.

Figure 10 (A-B) is a graph illustrating the effect of cocaine alone or analog **19** alone on locomotor activity in mice. The graph shows behavioral effects of cocaine (circles) and compound **19** (squares). Both **19** ($F_{4,58} = 12,65$, $P < 0.001$) and cocaine ($F_{5,65} = 15.63$, $P < 0.001$) produced significant and dose-dependent increases in the distance traveled (A) in male Swiss-Webster mice. Similarly, **19** ($F_{4,58} = 3.53$, $P < 0.05$) and cocaine ($F_{5,65} = 15.53$, $P < 0.001$) significantly increased stereotypic movements (B). The distance traveled and the stereotypic movement responses in vehicle control group were 2609 ± 233 cm and 1003 ± 103, respectively. *P < 0.05; ** P < 0.01; *** P < 0.001 as compared to the corresponding responses in the vehicle control group by Tukey's post hoc test.

Figure 10 (C-D) is a graph depicting the results of the discriminative stimulus testing in rats of of cocaine alone or analog **19** alone. The graph shows behavioral

effects of cocaine (circles) and compound **19** (squares). The results of the discriminative stimulus testing in rats (n = 7 to 10) are shown in panels C and D. The data points in the figure represent the mean ± S.E.M.

Figure 11 (A-B) is a graph illustrating the effect of cocaine alone or analog **20** alone on locomotor activity in mice. The Figure shows behavioral effects of cocaine (circles) and compound **20** (squares). Cocaine produced dose-dependent increases in the distance traveled ($F_{5,74}$ =: 30.5, $P < 0.001$) and stereotypic movements ($F_{5,74}$ = 8.3, $P < 0.001$) in male Swiss-Webster mice (A and B). Unlike cocaine, compound **20** produced small increases in the distance traveled and lacked stimulant effects on stereotypic movements (B). The distance traveled and the stereotypic movement responses in saline control group were 3517 ± 325 cm and 1299 ± 71 respectively. The corresponding numbers in the vehicle (10% DMSO) control group were 3230 ± 311 crn and 1197 ± 79, respectively. *P < 0.05; ** P < 0.01; *** P < 0.001 as compared to the responses in the corresponding vehicle control groups by Tukey's post hoc; test.

Figure 11 (C-D) is a graph depicting the results of the discriminative stimulus testing in rats of cocaine alone or analog **20** alone. The Figure shows behavioral effects of cocaine (circles) and compound **20** (squares). The discriminative stimulus testing in rats (n = 7 to 10) are shown in panels C and D. The data points in the figure represent the mean ± S.E.M.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

A lead compound according to the invention is a chemical compound selected for chemical modification to design analog compounds useful in the treatment of a given condition. The lead compound can be a known compound or a compound designed de novo.

A pharmacophore according to the invention is a chemical motif including a number of binding elements and their three-dimensional geometric arrangement. The elements are presumed to play a role in the activity of compounds to be identified as a lead compound. The pharmacophore will be defined by the chemical nature of the binding elements as well as the three-dimensional geometric arrangement of those elements.

The present invention includes compounds which are rationally designed to control dopamine flow in the brain. These compounds can be dopamine transporter

inhibitors and/or cocaine antagonists. Rational design of the compounds of the present invention includes identifying a mechanism associated with dopamine flow in the brain. Information relating to the mechanism is then analyzed such that compound structures having possible activity in interfering with such a mechanism are formulated. In particular, structures are synthesized based on "building blocks", wherein each building block has a feature potentially capable of interfering with a particular mechanism associated with dopamine flow, particularly, a mechanism mediated by dopamine transporter protein (DAT).

Compounds having different building block combinations are then synthesized and their activity in relation to the identified mechanism tested. Such tests are conducted in vitro and/or in vivo. The information obtained through such tests is then incorporated in a new cycle of rational drug design. The design-synthesis-testing cycle is repeated until a candidate compound having the desired properties for a targeted therapy; e.g. dopamine flow control, is obtained. The candidate compound is then clinically tested.

An approach for controlling dopamine flow in the brain for the treatment of cocaine addiction is to design cocaine antagonists which can affect dopamine uptake. More specifically, this approach is based on rationally designing compounds which are antagonists of cocaine which reduce or block cocaine binding to DAT. Preferably, antagonists are designed which reduce or block cocaine binding while leaving other aspects of dopamine transport unaffected. The designed antagonists should provide a basis for therapeutic protocols based on the selective control of dopamine transport and thereby control of synaptic signaling with no or little disruption of the normal flow of dopamine in the brain.

Although both cocaine and dopamine bind to the DAT, recent mutagenesis and pharmacokinetic studies provide evidence that dopamine and cocaine do not share an identical binding site on the DAT. Thus, one object of the present invention is to discover molecules that will compete with cocaine at its binding site, yet bind to the DAT in a manner that would not significantly inhibit the transport of dopamine. These molecules could potentially function as cocaine antagonists or as partial agonists if they bind in such a way that inhibition of dopamine uptake is incomplete. Such compounds would be useful to counter some of the adverse effects of cocaine in cases of cocaine overdose or help maintain patients in cocaine treatment program.

Recent advances in molecular biology have identified the amino acid sequences of the DAT, but no experimental 3D structures have been obtained for the DAT. The lack of experimental structures makes it difficult to employ a structure-based design strategy for the discovery of DAT inhibitors as cocaine antagonists.

On the other hand, a wealth of SAR data on cocaine analogs and other classes of dopamine transporter inhibitors are available. This makes it feasible to derive "putative 3D pharmacophore models", defined as the representation of crucial chemical structural features and their 3D geometric relationships that are important for the biological activity of interest. With the pharmacophore models, one can search large chemical databases to discover compounds whose 3D structures meet the pharmacophore requirements.

Using a lead compound identified according to the invention, a large number of DA inhibitors were designed and tested. Compounds have been identified which exhibit promising cocaine antagonism in a functional assay.

Identification of a pharmacophore for rational drug design of cocaine antagonists

In order to design a pharmacophore representing presumably key features in DAT inhibition, a number of functional groups shared by cocaine and its analog WIN-35065 have been considered. The chemical structures of cocaine and WIN-35065 are shown in Figure 1.

Based on extensive analysis of structure-activity relationships of cocaine and its analogs, three binding elements have been identified which are believed to play important roles in the binding and reuptake activities of cocaine and its analogs, (1) an aromatic system at the 30-position of the tropane ring; (2) a 2p ester group or a small hydrophobic group at this position; and (3) a nitrogen at position 8. The nitrogen at position 8 may be replaced by an oxygen.

The next step in formulating a pharmacophore based on the above binding elements is to determine the 3D geometric relationships of these binding elements in cocaine and its analogs and incorporating those relationships as geometric parameters, which will define the geometric requirements of the pharmacophore models.

In order to determine the geometric parameters for the design of a pharmacophore directed to cocaine based compounds, conformational analysis was performed on cocaine and WIN-35065. The X-ray crystal structure of cocaine was used as a starting point for modeling cocaine. The initial structure of WIN 35065 was

built by replacing the benzoyloxy group with a phenyl group using the QUANTA molecular modeling package. The structures of both compounds were minimized, and a systematic conformational search was performed, using the program QUANTA.

The binding elements described above were represented by a nitrogen atom, a carbonyl oxygen, and an aromatic ring, respectively.

In determining the geometric requirements of the pharmacophore, three distance parameters were defined: (i) the nitrogen and the oxygen; (ii) the distance between the nitrogen and the geometric center of the aromatic ring; and (iii) the distance between the oxygen and the geometric center of the aromatic ring. The ranges for these distance parameters were determined by generating conformational profiles of cocaine and WIN35065. The ranges were centered around the distance between two binding elements in cocaine and WIN-35065 conformations of low energy. The conformational profiles were then processed to determine the limits of each range.

Figure 2 shows the chemical structure and distance requirements of the pharmacophore employed in the identification of a lead compound for the design of compounds which can be useful in dopamine flow control, e.g., cocaine antagonists.

The distance requirements obtained for the pharmacophore of Figure 2 are: (i) a distance (d1) between the nitrogen and the oxygen of from 2.2 Å to 4.5 Å; (ii) a distance (d2) between the nitrogen and the geometric center of the aromatic ring of from 5.0 Å to 7.0 Å; and (iii) a distance 0 between the oxygen and the geometric center of the aromatic ring of from 3.4 Å to 6.1 Å. This essentially covers the possible span between these atoms in cocaine and WIN 35065. Some margin was allowed for both the lowest distance value (2.6 Å) and largest distance value (4.2 Å).

The limits of the distance ranges were selected in order to provide a fairly large distance tolerance. This stems from the consideration that while the identified lead compound should be based on the general structure of cocaine, for such lead compound to be useful in the design of cocaine antagonists the distance requirements of the pharmacophore should have sufficient flexibility such that compounds having diverse chemical structures can be identified. Such a broadly defined pharmacophore allows identification of compounds that not only effectively compete with cocaine binding to the DAT, but also may display different profiles by having a binding mode significantly different from that of cocaine and WIN-35065 compounds.

3D-Database Pharmacophore Search of the NCI 3D-Databases

Based on the pharmacophore model shown in Figure 2, the chemical structures of the 206,876 "open" compounds in the NCI 3D-database were analyzed with the program Chem-X. During the search process, a compound is first examined for the presence of the required binding elements, i.e., a secondary or a tertiary nitrogen, a carbonyl group, and an aromatic ring system. If the three binding elements are present in a compound retrieved from the data base, the program then investigates whether the compound has a conformation that meets the geometric requirements of the pharmacophore. Compounds having at least one conformation that met the distance requirements of the pharmacophore were selected for further processing. Up to 3,000,000 conformations were examined for each compound containing the three binding elements which define the pharmacophore.

Based on the pharmacophore model shown in Figure 2, a first group of compounds containing 4094 compounds, i.e., 2% of 206,876, was formed for further processing to identify a lead compound for rational drug design.

The first step in processing the compounds in the first group involved pruning the first group by eliminating all compounds having a molecular weight greater than 1000. This is to focus the drug design on smaller compounds having a limited number of sites to be modified.

The group of compounds were further pruned by eliminating compounds wherein the nitrogen atom in the pharmacophore is not capable of accepting a hydrogen bond; e.g., due to the chemical environment of the nitrogen atom in the compound.

Finally, in order to provide a relatively small number of compounds without sacrificing the structural diversity of the group of compounds obtained through the above two pruning steps, the compounds in the pruned group were distributed in clusters according to structural similarity, each cluster providing a class of compounds represented by one compound which was selected for the next step, i.e., in vitro testing. Based on the pruning steps described above, of the 4094 compounds identified according to the pharmacophore requirements, 385 compounds were finally selected for testing in [^{3}H]mazindol and [^{3}H]DA reuptake assays.

Screening of Compounds in [^{3}H] Mazindol and [^{3}H] DA Assays

In the first batch of screening, 70 compounds out of the 385 selected candidates were evaluated in the [^{3}H]mazindol binding assay. Thirteen compounds displayed more than 50% inhibition at 10 μM in the [^{3}H]mazindol binding assay. An additional 23 compounds showed an inhibitory activity of 30% to 50% at 10 p,M and 8 more compounds had an inhibitory activity of 20% to 30% at 10 μM in the [^{3}H]mazindol binding assay. Overall, 63% of 70 (44/70) compounds showed significant activity at 10 μM in the [^{3}H]mazindol assay. These results show that the pharmacophore model used in the 3D pharmacophore search was unexpectedly effective in identifying compounds with diverse chemical structures that can effectively compete with [^{3}H]mazindol binding to the cocaine site on the DAT.

The group of compounds having DAT binding activity were further tested for their ability to antagonize cocaine's inhibition of [^{3}H]DA uptake. Four classes of compounds were found to display significant functional antagonism. One such class is represented by compound **3,** the structure of which is shown on Figure 3. Compound **3** was found to have good potency in DAT binding and uptake assays with K_i values of 550 nM and 330 nM, respectively, only slightly less potent than cocaine. Importantly, this compound has a simple chemical structure, which greatly facilitates subsequent structure-activity relationship studies.

Based on the above data, compound **3** was selected as a lead compound for the rational design of compounds capable of altering dopamine flow in the brain, for example, by acting as cocaine antagonists.

The rational drug design of compounds based on lead compound **3** is discussed below.

In order to determine a strategy for modifying compound **3,** the functional antagonism of **3** in terms of its effects on the inhibition of [^{3}H]dopamine uptake by cocaine was first determined. The results are shown in Table 1 and Figure 4.

The IC_{50} values of cocaine in the presence of **3** were determined and compared to the IC_{50} value of cocaine alone. Significant differences in IC_{50} values were found and compared to theoretical IC_{50} values expected from models of "same site" antagonism. As can be seen from Table 1 and Figure 4, at each of the three concentrations of **3** used in the tests (50, 200 and 500 nM), the IC_{50} value obtained for cocaine was significantly greater than that expected from "same site" antagonism.

Therefore, 3 was considered to have significant functional antagonism against cocaine, though it is important to point out that at these concentrations **3** significantly inhibited transport alone and that the data are normalized to 100% for the analysis.

Since cocaine is a potent inhibitor of not only DA uptake, but also of 5-HT and NE uptake, the activity of compound 3 as an inhibitor of these transporters was also tested. The results are shown in Table 2. As can be seen, compound 3 is more selective for DAT than is cocaine.

Furthermore, the ability of compound 3 to stimulate locomotor activity in mice was examined. These tests have shown that compound 3 failed to stimulate locomotor activity at concentrations from 1.0 mg/kg to 30 mg/kg.

Taken together, based upon its fairly potent activities in binding and uptake, its simple chemical structure, its profile at the three different transporter sites, its functional antagonism and its inability to stimulate locomotor activity in mice, compound 3 represent a promising lead compound for further development and rational drug design.

Table 1. Functional antagonism of **3** against cocaine

Drugs	IC_{50} (nM) [^{3}H]-Dopamine Uptake (Experimental)	IC_{50} (nM) [^{3}H]-Dopamine Uptake (Theroretical, assuming one binding site for cocaine and the drug)
Cocaine alone	297 ± 22[a]	
Cocaine + **3** (50 nM)	470 ± 25	331 ± 11
Cocaine + **3** (200nM)	717 ± 49	438 ± 22
Cocaine + **3** (500nM)	1161 ± 100	652 ± 24

[a] Standard error was based on three experiments

Designs of analogs of 4-hydroxy-1 methyl-4 (4-methylphenyl)-3 piperidyl 4-methylphenyl ketone

In order to investigate the structure-activity relationships of analogs of compound **3**, such analogs were designed, synthesized and tested. A total of 25

analogs, including the lead compound **3,** were synthesized in racemic form and tested as inhibitors at the DAT site for their uptake activities. For a number of potent analogs, their binding affinities to the DAT were measured and were also evaluated as inhibitors at the NET and SERT sites. The structures of compound **3** and its analogs **4** through **27** are also shown in Tables 2A. and 2B., and their activities are summarized in Table 3.

Compounds 6 through 11:

The general synthesis of the piperidinol analogs **6-11** is illustrated in Figure 5. Briefly, reaction of methylamine hydrochloride with an excess of aryl methyl ketone and paraformaldehyde in the presence of a catalytic amount of acid led to compounds **3, 6,** and **7.** The 1,3-dihydroxy compounds (**8, 9** and **10**) were synthesized by reducing the appropriate ketones with DIBAL-H in THF at -78 °C.

Under these conditions, the reduction led to the cis-stereoisomers **8, 9** and **10.** The cyclic carbonate **11** was prepared by the treatment of compound **8** with triphosgene in DCM. Compounds **4** and **5** were purchased from commercial suppliers.

As can be seen, in Tables 2A., 2B., and 3, analog **4** without the methyl group on each aromatic ring has binding and uptake activities reduced by 9- and 7-fold, respectively, as compared to the lead compound **3.** Replacement of the methyl group on each aromatic ring by a fluorine atom (**5**) reduces the binding and uptake activities each by 12-fold. Our molecular modeling studies showed that **4, 5** and the lead compound (**3**) have essentially the same conformational profiles.

Therefore, it is believed that the activity difference between compounds **4** and **5** on one hand and compound **3** on the other hand, may be primarily due to the difference in their hydrophobicity. Accordingly, compounds **6** and **7** were designed and synthesized to obtain analogs having a higher hydrophobic character than compound **3.** Rationally designed analog **6** bearing 3,4-dichloro substitutions has a much improved activity in both binding and uptake. Its binding affinity to the DAT was increased by 45-fold and its uptake activity at the DA site was increased by 7-fold.

Furthermore, compound **6** showed higher selectivity for the DA site relative to the 5-HT and NE sites. While compound **6** has a relatively potent activity at the NE site, it has a very weak activity at the 5-HT site.

In sharp contrast, analog **7** with 2,4-dicholoro substitutions has a much-reduced activity in binding and uptake, compared to compound **6**. The DAT binding affinity of compound **7** was reduced by 7-fold, while its uptake activity at the DA site was reduced by as much as 16-fold, compared to compound **6**.

Thus, despite the similar hydrophobicity of **6** and **7**, their binding affinities to the DAT differ by 335-fold and their uptake activities at the DA site differ by 115-fold.

The above tests show that in addition to hydrophobicity, other factors, such as the conformational differences between **6** and **7**, may play an important role in their binding and uptake activities. Therefore, a rational drug design approach was centered on chemically modifying compound **3** to generate analogs having a predetermined conformational profile.

In this regard, constrained compound **11** was designed and synthesized to investigate further the active conformation for this class of compounds. In comparison to the lead compound (**3**), the binding and uptake activities of **11** were reduced by 17-fold and 26-fold, respectively.

Yet another avenue for designing analogs of compound **3** focuses on chemically modifying compound **3** to modify the carbonyl binding element of the pharmacophore. To investigate the importance of this carbonyl group, we have reduced this carbonyl group to a hydroxyl group in the lead compound **3**, and analogs **6** and **7**, which resulted in compounds **8, 9, 10**, respectively. As compared to the lead compound **3**, the binding affinity for **8** was reduced by 22-fold and the uptake activity was reduced by 44-fold, highlighting the importance of the carbonyl group. The binding and uptake activities for **9** were reduced even more, by 380- and 83-fold, respectively, as compared to the corresponding compound **6**.

On the other hand, there is only a slight reduction of the activities for **10** as compared to **7**, probably because both compounds have only weak activities.

The data presented in Tables 2A. , 2B. and 3 suggest that the carbonyl group in this class of compounds is important for their activity. Because both carbonyl and hydroxyl groups can function as a hydrogen bonding acceptor, the diminished activity in the corresponding hydroxyl compounds (**8**, **9** and **10**) may be primarily due to the change in conformational profiles of these compounds.

Compounds **12** through **27**:

The general synthesis of the compounds **12-27,** using known methods from the literature, is illustrated in Figure 6. Scheme 2. Briefly, reaction of an amine hydrochloride with an excess of an aryl methyl ketone **(29-44)** and paraformaldehyde in the presence of a catalytic amount of hydrochloric acid led to compounds **12-27** (Figure 5. Scheme 2). All substituted acetophenones were purchased from commercial suppliers, except compound **29**, which was prepared in 90% yield by reacting the substituted phenacyl chloride **28** with $SnCl_2$ and NaI in a 5:1 mixture of THF and H_2O for 2 hours (Figure 6. Scheme 1).

Our preliminary structure-activity relationship studies suggested that the substituents on each phenyl ring have considerable influence on their binding and uptake activities. To gain a better understanding of the effects of phenyl substitutions, analogs **12-20** were made and tested. Lead compound **3** with a *p*-methyl substituent in both phenyl rings has K_i values of 492 and 360 nM against [^{3}H]mazinol binding and DA uptake, respectively. A *p*-fluoro substituent in both phenyl rings resulted in analog **6** having K_i values of 5700 and 4200 nM in binding and inhibition of DA reuptake, respectively. Therefore, analog **6** is approximately 12-fold less potent than **3** in both assays, suggesting that substituent at the para position of both phenyl rings has a significant effect on binding to the transporter. To investigate further this effect, analogs **12-15** were synthesized and evaluated. A *p*-chloro substituent in both phenyl rings resulted in analog **12** with K_i values of 254 and 160 nM in binding and inhibition of DA reuptake, respectively, approximately 2-times more potent than **3** in both assays. Compared to the unsubstituted analog **5,** the *p*-chloro substituent in both phenyl rings improved the binding and inhibition of DA reuptake activities by 17- and 16-fold, respectively. Analog **13** with a *p*-bromo substituent in both phenyl rings has K_i values of 231 and 85 nM in binding and inhibition of DA reuptake, representing 19- and 30-fold improvement compared to the unsubstituted analog **5**. A *p*-iodo substituent in both phenyl rings resulted in analog **14**, with a K_i value of 124 nM in inhibition of DA uptake, a 21-fold improvement compared to the unsubstituted analog **5** but slightly less potent than **13** with a *p*-bromo substituent in both phenyl rings. Analog **15** with a *p*-ethyl substituent in both phenyl rings has a K_i value of 8090 nM in inhibition of DA uptake, a 22-fold loss in affinity as compared to the lead compound **3** with a *p*-methyl substituent. Therefore, a bromo atom appears to be optimal to achieving the highest potency in inhibition of DA reuptake among analogs

3, 5, 6, 12, 13, 14 and **15** with a mono substituent at the para position in both phenyl rings.

To investigate the effect of the substitution positions (ortho, meta and para) in the phenyl rings, two analogs, **16** and **17** with a mono chloro substituent at either the ortho or meta position in both phenyl rings were synthesized, evaluated and compared to analog **12** with a chloro substituent at the para position in both phenyl rings. Analog **16** with a chloro substituent at the ortho position in both phenyl rings has K_i values of 23180 and 23340 nM in binding and inhibition of DA uptake, respectively, 91- and 65-fold less potent than analog **12**. Analog **17** with a chloro substituent at the meta position in both phenyl rings has K_i values of 157 and 155 nM in binding and inhibition of DA uptake, comparable to the activities of analog **12** in both assays. Since **12, 16** and **17** have very similar hydrophobicity, the difference between **12, 16** and **17** in their activities in binding and inhibition of DA reuptake is likely due to their difference in conformational profiles and this has been further investigated through our molecular modeling studies (see Molecular Modeling section).

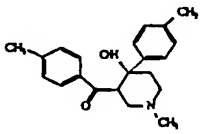
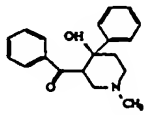
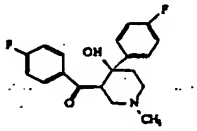
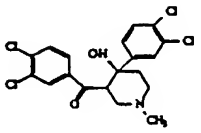
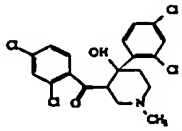
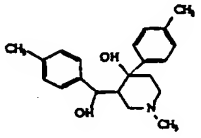
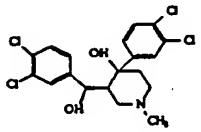
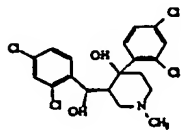
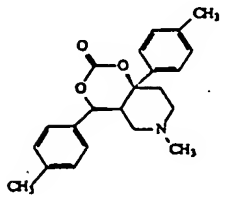
Earlier structure-activity relationship studies led to the identification of a potent analog (**4**) with 3,4-dichloro substituents in both phenyl rings. To explore further the structure-activity relationships of compounds with two substituents in both phenyl rings, analogs **18, 19** and **20** were made and tested. Analog **18** with 3, 4-difluoro substituents has a K_i value of 888 nM in the inhibition of DA uptake, a 5-fold improvement as compared to the 4-fluoro, mono-substituted analog **6** but 17-times less potent than the 3, 4-dichloro substituted analog **4**. Analog **19** with 3,4-dimethyl substituents has K_i values of 304 and 101 nM in binding and inhibition of DA uptake. It is interesting to note that as compared to the 3,4-dichloro substituted analog **4,** the activity of **19** in inhibition of the reuptake of DA is only 2-fold less potent, but the binding affinity for the [^{3}H]mazindol site is reduced by 28-fold. Analog **20** with 4-chloro-3-methyl-substituents has K_i values of 59 and 112 nM in binding and inhibition of DA uptake, 5- and 2-fold less potent than **4** in its binding and uptake activities, respectively.

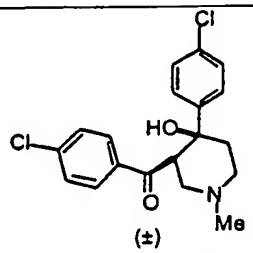
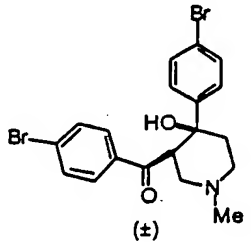
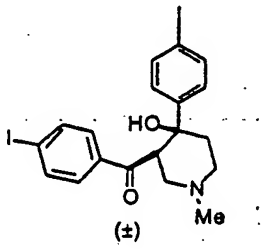
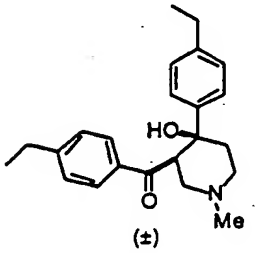
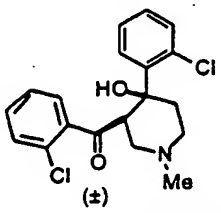
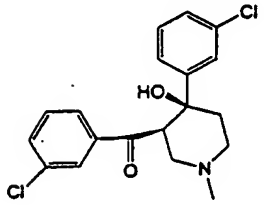
Previous investigations of a tropane series of compounds showed that fairly large *N*-substituents may be tolerated for their activity in inhibition of DA reuptake. To investigate the effect of *N*-substitutions in this class of DAT inhibitors, a series of analogs were synthesized and tested (**21-27**). In contrast to the structure-activity relationship of tropane analogs in general, replacement of the *N*-methyl group with

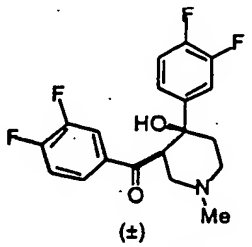
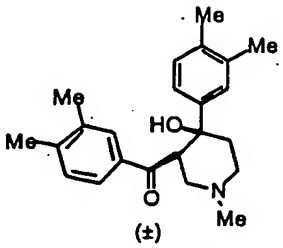
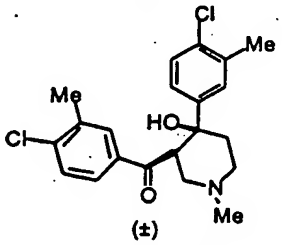
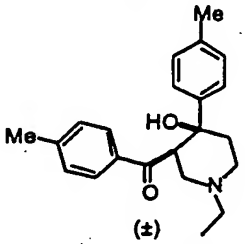
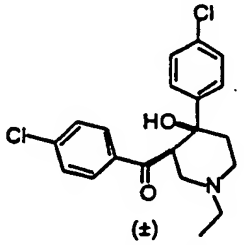
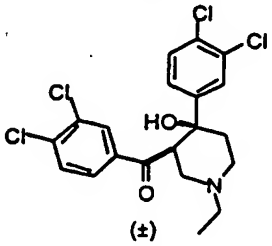
larger substituents decreases the activity in inhibition of the reuptake of DA in this class of compounds. Analog **21** with a *N*-ethyl substituent and a 4-methyl substituents at both phenyl rings has a K_i value of 1144 nM in inhibition of DA uptake, 3-fold less potent than its corresponding *N*-methyl analog **3**. Analog **22** with a *N*-ethyl substituent and a 4-Cl substituent at both phenyl rings has a K_i value of 574 nM in inhibition of DA uptake, 4-fold less potent than its corresponding *N*-methyl analog **12**. The only exception is analog **23** with a *N*-ethyl substituent and 3,4-dichloro substituents in both phenyl rings, which has a K_i value of 52 nM in inhibition of DA uptake, as potent as its corresponding *N*-methyl analog **4** (K_i equal to 51 nM). With larger *N*-substituents, the potency of the analogs decreases even more. Analog **24** with a *N*-ethylphenyl substituent and 3,4-dichloro substituents in both phenyl rings has a K_i value of 389 nM, 8-fold less potent than its corresponding *N*-methyl analog **4**. Analog **25** with a *N*-ethylphenyl substituent and a 4-bromo substituent in both phenyl rings has a K_i value of 1492 nM, 18-fold less potent than its corresponding *N*-methyl analog **13**. Analog **26** with a *N*-propylphenyl substituent and 3,4-dichloro substituents in both phenyl rings has a K_i value of 500 nM, 10-fold less potent than its corresponding *N*-methyl analog **4**. Analog **27** with *N*-propylphenyl substituent and a 4-bromo substituent in both phenyl rings has a K_i value of 1399 nM, 17-fold less potent than its corresponding *N*-methyl analog **13**. Therefore, it appeared that a *N*-methyl substituent is optimal for the reuptake activity with this class of compounds.

Cocaine potently inhibits the reuptake of DA, but even more potently inhibits the reuptake of serotonin (5-HT) and norepinephrine (NE) (Table 3). To assess the selectivity of several potent analogs (**12, 13, 17, 19, 20, 23**), we evaluated them as reuptake inhibitors at SERT and NET sites and the results are summarized in Table 3. The selectivity between DAT relative to SERT for analogs **4,12,13,17,19, 20** and **23** is 47, 6, 22, 3, 8, 10, and 43-fold, respectively. Thus, analogs **4** and **23** have the best selectivity between DAT and SERT, being 47- and 43-fold, respectively. The selectivity between DAT and NET for analogs **4, 12, 13, 17, 19, 20** and **23** is 4, 8, 22, 3, 8, 6 and 11-fold, respectively. Therefore, in contrast to cocaine, in general, these inhibitors are more potent at the DAT site among these three transporter sites.

Table 2A. Chemical structures of piperidinols.

Compound	Compound	Compound
	3	4-Hydroxy-l-methyl-4-(4-methylphenyl)-3-piperidyl 4-Methylphenyl Ketone
	4	4-Hydroxy-l-methyl-4-phenyl-3-piperidyl phenyl Ketone
	5	4-Fluorophenyl-4-hydroxy-1-methyl-3-piperidyl 4-fluorophenyl Ketone
	6	3,4-Dichlorophenyl 4-(3,4-Dichlorophenyl)-4-hydroxy-l-methyl-3-piperidyl Ketone
	7	2,4-Dichlorophenyl 4-(2,4-Dichlorophenyl)-4-hydroxy-l-methyl-3-piperidyl Ketone
	8	3 -[Hydroxy(4-methylphenyl)methyl]-1-methyl-4-(4-methylphenyl)piperidin-4-ol
	9	4-(3,4-Dichlorophenyl)-3-[(3,4-dichlorophenyl)hydroxymethyl] -1-methylpiperidin-4-ol
	10	4-(2,4-Dichlorophenyl)-3-[(2,4-dichlorophenyl)hydroxymethyl] -1-methylpiperidin-4-ol
	11	8-Aza-1,5-bis(4-methylphenyl)-8-methyl-2,4-dioxabicyclo[4.4.0]decan-3-one

Compound	Compound	Compound
	12	4-Chlorophenyl4-(4-Chlorophenyl)-4-hydroxy-l-methyl-3-piperidyl Ketone
	13	4-Bromophenyl4-(4-Bromophenyl)-4-hydroxy-l-methyl-3-piperidyl Ketone
	14	4-Hydroxy-4-(4-iodophenyl)-1-methyl-3-piperidyl 4-iodophenyl Ketone
	15	4-Ethylphenyl4-(4-Ethylphenyl)-4-hydroxy-l-methyl-3-piperidyl Ketone
	16	2-Chlorophenyl 4-(2-Chlorophenyl)-4-hydroxy-l-methyl-3-piperidyl Ketone
	17	3-Chlorophenyl 4-(3-Chlorophenyl)-4-hydroxy-l-methyl-3-piperidyl Ketone

Compound	Compound	Compound
	18	3,4-Difluorophenyl 4-(3,4-Difluorophenyl)-4-hydroxy-1-methyl-3-piperidyl Ketone
	19	3,4-Dimethylphenyl 4-(3,4-Dimethylphenyl)-4-hydroxy-1-methyl-3-piperidyl Ketone
	20	4-Chloro-3-methylphenyl 4-(4-Chloro-3-methylphenyl)-4-hydroxy-1-methyl-3-piperidyl Ketone
	21	1-Ethyl-4-hydroxy-4-(4-methylphenyl)-3-piperidyl 4-Methylphenyl Ketone
	22	4-Chlorophenyl 4-(4-Chlorophenyl)-1-ethyl-4-hydroxy-3-piperidyl Ketone
	23	3,4-Dichlorophenyl 4-(3,4-Dichlorophenyl)-1-ethyl-4-hydroxy-3-piperidyl Ketone

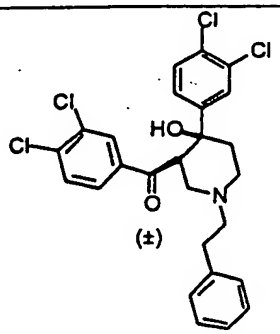
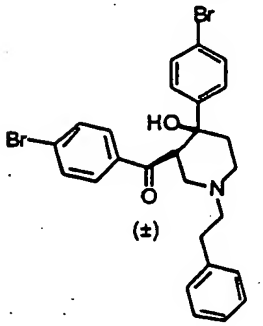
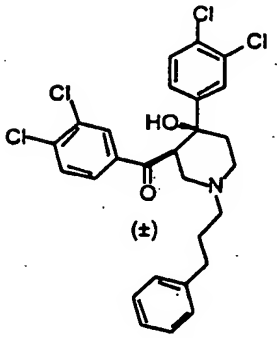
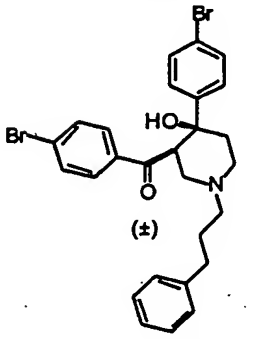
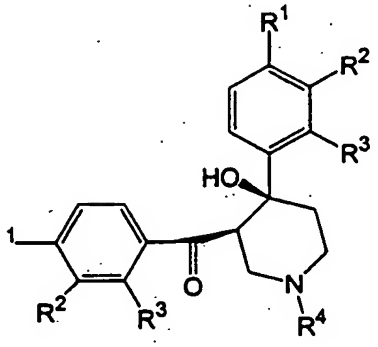
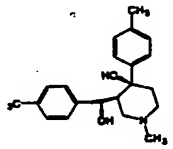
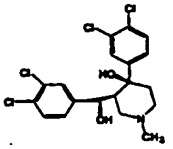
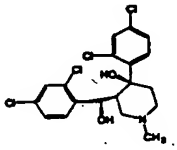
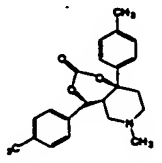
Compound	Compound	Compound
	24	3,4-Dichlorophenyl 4-(3,4-Dichlorophenyl)-4-hydroxy-l-(2-phenylethyl)-3-piperidyl Ketone
	25	4-Bromophenyl 4-(4-Bromophenyl)-4-hydroxy-l-(2-phenylethyl)-3-piperidyl Ketone
	26	3,4-Dichlorophenyl 4-(3,4-dichlorophenyl)-4-hydroxy-l-(3-phenylpropyl)-3-piperidyl Ketone
	27	4-Bromophenyl 4-(4-Bromophenyl)-4-hydroxy-l-(3-phenylpropyl)-3-piperidyl Ketone

Table 2B. Chemical structures of piperidinols



Compound	R^1	R^2	R^3	R^4
3	Me	H	H	Me
4	Cl	Cl	H	Me
5	H	H	H	Me
6	F	H	H	Me
7	Cl	H	Cl	Me
8				
9				
10				
11				

12	Cl	H	H	Me
13	Br	H	H	Me
14	I	H	H	Me
15	Ethyl	H	H	Me
16	H	H	Cl	Me
17	H	Cl	H	Me
18	F	F	H	Me
19	Me	Me	H	Me
20	Cl	Me	H	Me
21	Me	H	H	Ethyl
22	Cl	H	H	Ethyl
23	Cl	Cl	H	Ethyl
24	Cl	Cl	H	$(CH_2)_2Ph$
25	Br	H	H	$(CH_2)_2Ph$
26	Cl	Cl	H	$(CH_2)_3Ph$
27	Br	H	H	$(CH_2)_3Ph$

Table 3. The activities and selectivity of racemic compounds at three mono-amine transporter sites.

Compound	K_i (nM)				Selectivity	
	Binding ([^{3}H]-Mazindol)	Uptake ([^{3}H]-DA)	([^{3}H]-SER)	([^{3}H]-NE)	5-HT DA	NE DA
I[b] (cocaine	231± 22[a]	274 ± 20	155 ± 0.4	108 ± 4	0.6	0.4

3[b]	492 ± 34	360 ± 25	1630 ± 150	3860 ± 70	4.5	10.7
4[b]	10.9 ± 1.4	51 ± 8	2380 ± 140	177 ± 49	46.7	3.5
5[b]	4420 ± 620	2590 ± 230				
6[b]	5700 ± 360	4200 ± 70				
7[b]	3650 ± 170	5850 ± 1080				
8[b]	10650 ± 650	15800 ± 650				
9[b]	4140 ± 60	4220 ± 20				
10[b]	4350 ± 80	7520 t 90				
11[b]	8190 ± 30	9480 ± 740	1270 ± 110	17240 ± 740	0.1	1.8
12	254 ± 6	160 ± 16	928 ± 7	1210 ± 60	5.8	7.6
13	231 ± 46	85 ± 19	1880 ± 190	1860 ± 90	22.1	21.9
14		124 ± 1				
15		8090 ± 530				
16	23180 ± 90	23340 ± 1063				
17	157 ± 5	155 ± 5	456 ± 44	434 ± 83	2.9	2.8
18		888 ± 95				
19	304 ± 25	101 ± 12	811 ± 10	1014 ± 91	8.0	10.0
20	59 ± 13	112 ± 15	1160 ± 110	640 ± 61	10.4	5.7
21		1144 ± 32				
22		574 ± 28				
23	40.5 ± 0.5	52 ± 4	2220 ± 190	610 ± 108	42.7	11.7
24		389 ± 4				
25		1492 ± 5				
26		500 ± 16				
27		1399 ± 154				

[a]Mean ± standard error or range of 2-3 experiments, each conducted using six concentrations of drug in triplicate.

[b]Data taken from reference 54.

Structure-Activity Relationships of Enantiomers and Their Selectivity at the Three Transporter Sites

Each of the compounds shown in Table 2A were synthesized in racemic form and the results are somewhat complicated by the presence of two stereoisomers. To solve this problem, the individual enantiomers of 4 potent analogs, including **3** (the lead compound), **4, 19** and **20** were obtained through chiral HPLC separation (see Experimental Section). These enantiomers were then evaluated as inhibitors at all the three transporter sites and the results are summarized in Table 4.

It has been unexpectedly discovered that the (-)-isomers are more potent inhibitors than the (+)-isomers at the DAT site with each compound. Thus, the (-) isomer for each compound has an activity in inhibition of DA reuptake approximately 2-fold greater than its respective racemic form. The difference in activities between the (+) and (-) isomers in inhibition of DA reuptake is fairly large for **3** and **19**, being 7.6- and 10.8-fold, respectively, but the difference is relatively small for **4** and **20**, being only 1.4- and 2.5-fold, respectively. Comparison between their structures showed that **3** and **19** have a *para*-methyl substituent in each phenyl ring, while **4** and **20** have a *para*-Cl substituent in each phenyl ring. Replacement of the para-methyl group in **19** with the *para*-Cl group resulted in **20**. The improvement in activities in inhibition of DA reuptake is 7.0-fold from (+)-**19** to (+)-**20** but is only 1.6-fold from (-)-**19** to (-)-**20**. These results suggested that (+) and (-) isomers may bind to the DAT in different manner and a *para*-Cl group in each phenyl ring more significantly improves the potency for the (+) isomers than for the (-) isomers.

Furthermore, the (+) and (-) isomers have different selectivity profiles among the three transporters, especially for **3** and **19**. While (+)-3 is only marginally selective for DAT, (-)-**3** is quite selective for DAT, with a selectivity of 7.3-fold between DAT and SERT sites and 33.7-fold between DAT and NET sites. Analog (+)-**19** isomer is in fact more selective for SERT, but (-)-**19** is fairly selective for DAT. Both the (+) and (-) isomers of **4** and **20** are selective for DAT, suggesting the *para*-Cl substitutent in each phenyl ring in these two compounds is a favorable binding element for the DAT and/or an unfavorable binding element relative to the SERT and NET sites.

Molecular Modeling Studies

To confirm the above hypotheses, the following molecular modeling experiments were conducted.

Compounds **3** through **11**:

Apart from occasional replacement of the carbonyl group with a hydroxyl, each of the compounds in Tables 2A, 2B and 3 contains the pharmacophore as shown in Figure 1, yet they display significantly different activities. The most potent compound **6** has a binding affinity of 11 nM, while the least potent compound **8** has a binding affinity of 11 μM, a difference of 3 orders of magnitude. Therefore, it is believed that in addition to the pharmacophore, other factors also play important roles in the binding and uptake activities for this class of compounds to the DAT.

To gain a better understanding on the binding of this class of compounds to the DAT, extensive conformational analysis has been conducted.

As shown in Figures 8A to 8D, comparison of the low energy conformational clusters of lead compound (**3**), and analogs **4, 5** and **6** showed that these compounds have virtually identical conformational profiles (Figure 7A), suggesting that the differences in their binding and uptake activity are not due to their conformational difference. In fact, their binding and uptake activities have a good correlation with their hydrophobicity, i.e., the most hydrophobic compound **6** has the most potent binding and uptake activities while the least hydrophobic compound **4** is the least potent. However, it is clear that the hydrophobicity is not the only factor important for the binding and uptake activities of this class of compounds. Compound **7** is more than 300-fold less potent than **6** in binding affinity, despite their similar hydrophobicity.

Conformational analysis showed that **7** has a different conformational profile as compared to the lead compound **3** and analog **6**. Although the piperidinyl ring and the phenyl ring at position **4** adopt identical orientation in the lowest energy conformations and other low energy conformations of **3, 6** and **7**, the phenyl ring-connected to the carbonyl group in the lowest energy conformation of **7** deviates by 39 from the phenyl ring in the lowest energy conformations of **3, 4** and **6** (Figure 7B). To achieve the same orientation, **7** would have to pay an energy penalty of **6** kcal/mol. These data suggest that in addition to their hydrophobicity, conformational preference of these compounds plays a crucial role for their binding and uptake activities.

The importance of the conformational preference is highlighted by 9. Despite its similar hydrophobicity to **6, 9** is 380-fold less potent than **6** in its binding affinity. Indeed, conformational analysis showed that the lowest energy conformation of **9** is significantly different from that of **3** and **6** (Figure 7C). In order to adopt the same conformation as the lowest energy conformation of **6, 9** would have to pay an energy penalty of as much as **8** kcal/mol. Rigid analog **11** is 751-fold less potent than **6** upon comparison of their binding affinities. Conformational analysis showed that the lowest energy conformation of **11** is significantly different from that of **6**. In fact, the corresponding phenyl rings at position **3** deviate as much as 94° (Figure 7D) and no low energy conformations of **11** are similar to the lowest energy conformation of **6**.

In summary, the molecular modeling studies for analogs **3-11** showed that in addition to hydrophobicity, the conformational preferences of the class of compounds designed based on lead compound **3** play an important role in determining their binding and uptake activities. The data suggest that the lowest energy conformation of **3, 4, 5** and **6** may represent the biologically active conformation for this class of compounds in binding to the DAT.

Compounds **12** through **27**:

Our structure-activity relationship studies showed that the nature and the position of the substituent(s) on both phenyl rings play an important role in the binding and reuptake activities for this class of compounds. To achieve a better understanding of factors important to their activities and to guide our further design of new analogs, we have carried molecular modeling studies. Because we have much more extensive structure-activity relationships with the racemic compounds, the structure-activity relationship of the racemic compounds at the DAT site was used in the present molecular modeling study. Based upon the data obtained for 8 chiral compounds (Table 4), the (-) isomer is always the more active compound between the two isomers and its activity is approximately two-times more potent than its racemic form. Thus, the molecular modeling results based upon the structure-activity relationships of the racemic compounds are valid for the (-)-isomers but not for the (+)-isomers.

Analogs **12, 16** and **17** all have a mono chloro substituent on each phenyl ring. While **12** and **17** have similar activities in inhibition of the reuptake of DA (160 nM and 155 nM in K_i, respectively), **16** (23340 nM in K_i) is approximately 150-fold less potent than **12** and **17**. Since **12, 16 and 17** only differ in the position of the chloro

substituent in both phenyl rings and have similar size and hydrophobicity, the substantial difference in their activities is thus likely due to their conformational preferences. Conformational analysis showed that the lead compound **3**, analogs **12** and **17** have virtually identical conformational profiles. They have essentially identical lowest energy conformations (Fig. 9A) and also have similar low minimum conformations. However, analog **16** has a significantly different conformational profile from **3, 12** and **17**. In its lowest energy conformation of **16**, the phenyl ring attached to the carbonyl group adopts a significantly different orientation from that in **3, 12** and **17**, deviated by approximately 40° (Fig. 9B). In order to adopt the same orientation as that in **3, 12** and **17**, analog **16** would have to pay an energy penalty as much as 9 kcal/mol. Therefore, although it is not absolutely certain that the lowest energy conformations of **3, 12** and **17** must be their active conformation in binding to the DAT, our results strongly suggest that conformational preference of these analogs plays an important role for the activity in inhibition of the reuptake of DA for this class of compounds.

Through our structure-activity relationship studies, it was found that analogs with a mono substituent at the para position in both phenyl rings have significantly different activities in inhibition of the reuptake of DA. For example, while **13** with a *p*-bromo substituent has a K_i value of 85 nM in inhibition of DA reuptake, **15** with a *p*-ethyl substituent has a K_i value of 8090 nM, 95-fold less potent than **13**. Conformational analysis of these compounds showed that they have virtually identical conformational profiles (data not shown), suggesting that their difference in activity may not be due to their conformational preference. Examination of these substituents showed that they differ in size and hydrophobicity, among other parameters. To investigate if their size and hydrophobicity play a role in determining their activity in inhibition of the reuptake of DA, we calculated the size (volume, V_m) and hydrophobicity (partition coefficient between n-octanol and water, logP) value for each analog, as well as their difference in size (ΔV_m) and hydrophobicity (ΔlogP), as shown in Table 5.

Using the genetic function approximation algorithm implemented in the Cerius2 program, several quantitative models were obtained. A strong parabolic correlation (eq. (1)) was found between the K_i for reuptake of DA and the size of the substituents in the phenyl rings for these compounds. The optimal value is approximately equal to the size of a bromo group.

$$\log(\frac{1}{K_i}) = 5.18 + 0.095\,\Delta V_m - 0.0014(\Delta V_m)^2 \quad (1)$$

$r^2 = 0.78, F = 7.18$

The correlation between logP and the K_i for reuptake of DA is however only marginal ($r^2 = 0.25$, eq. (2)).

$$\log(\frac{1}{K_i}) = 2.20 + 1.83\log P - 0.191(\log P)^2 \quad (2)$$

$r^2 = 0.25, F = 0.67$

When combined logP and ΔV_m, a stronger correlation was obtained ($r^2 = 0.89$, eq. (3)).

$$\log(\frac{1}{K_i}) = 3.61 + 0.70\log P + 0.051\Delta V_m - 0.0011(\Delta V_m)^2 \quad (3)$$

$r^2 = 0.89, F = 8.18$

Thus, the size of the substituents plays an important role in determining their potency in inhibition of the reuptake of DA for analogs with a mono substituent at the para position in both phenyl rings, while the hydrophobicity (logP) play a less significant role. Taken together, the results obtained from current and previous studies indicated that for this class of compounds, the pharmacophore, the conformational preference and the substituent size in the phenyl rings play important roles, while their hydrophobicity play some but less significant role in determining their affinity for the DA reuptake site.

Table 4. Activities of chiral isomers of several potent inhibitors at the three transporter sites.

Compound #	K_i (nM)				Selectivity	
	Binding ([^{3}H]-Mazindol)	Uptake ([^{3}H]-DA)	([^{3}H]-5-HT)	([^{3}H]-NE)	5-HT / DA	NE / DA
1[b] (cocaine	231 ± 22[a]	274 ± 20	155 ± 0.4	108 ± 4	0.6	0.4
(+)-**3**	1925 ± 203	1641 ± 223	617 ± 27	5170 ± 440	2.5	2.9

(-)-**3**	274 ± 5	216 ± 22	1581 ±19	7270 ± 1320	7.3	33.7
(+)-**4**	20 ± 2	51 ± 7	1118 ± 95	377 ± 38	21.9	7.4
(-)-**4**	13 ± 2	37 ± 2	895 ± 88	180 ± 4	24.2	4.9
(+)-**19**	1570 ± 2000	779 ± 131	372 ± 4	3550 ± 70	0.5	4.6
(-)-**19**	60 ± 3	72 ± 5	493 ± 52	778 ± 77	6.9	10.8
(+)-**20**	82 ± 14	111 ± 7	973 ± 85	545 ± 42	8.8	4.9
(-)-**20**	22 ± 5	45 ± 8	930 ± 50	519 ± 71	21	11

[a]Mean ± standard error or range of 2-3 experiments, each conducted using six concentrations of drug in triplicate.

Table 5. Calculated hydrophobicity (logP) and volume of para, mono-substituted analogs.

Analog	Substitue nt	-log K_i	Vm	ΔVm	LogP	ΔLogP
5	H	5.59	285.95	0.00	2.42	0.00
3	CH_3	6.44	319.12	33.17	3.35	0.93
6	F	5.3 8	295.26	9.31	2.70	0.27
12	C_l	6.80	312.73	26.78	3.46	1.03
13	Br	7.07	322.35	36.41	4.00	1.58
14	1	6.91	334.03	48.08	4.93	2.51
15	CH_3CH_2	5.09	353.48	67.53	4.15	1.73

Functional Antagonism

The original lead compound (±)-**3** was found to have a significant functional antagonism against cocaine in inhibition of the reuptake of DA. A DAT inhibitor with a significant functional antagonism suggests that the inhibitor is capable of reducing the binding of cocaine, either by direct steric hinderance or by an allosteric mechanism, while at the same time having a relatively lesser effect on DA binding.

It was found that (±)-**3** displays a significant antagonism at each of the three concentrations tested (50,200 and 500 nM). Since the functional antagonism of **3** was originally obtained with a racemate, it was not clear which chiral isomer is responsible for the observed functional antagonism and it is desirable to evaluate the enantiomers in the functional antagonism assay. At concentrations of 50 and 200 nM of (±)-**3**, (+)-**3** and (-)-**3** isomers presumably have 25 and 100 nM each in the mixture. At these concentrations, only (-)-**3** has a significant activity in binding to DAT, while (+)-**3** has a minimal activity. Hence, it is predicted that the functional antagonism of (±)-**3** may be primarily due to the (-)-**3** isomer. To confirm this, we evaluated the functional antagonism of (-)-**3**. Since (-)-**3** is approximately 2-times more potent than (±)-**3** in inhibition of DA uptake, we tested the functional antagonism of (-)-**3** at 30 and 100 nM in order to compare directly with the functional antagonism of (±)-**3** obtained at 50 and 200 nM. The results are summarized in Table 6. As can be seen, (-)-**3** and (±)-**3** have similar functional antagonism at concentrations that produce similar inhibition of DA reuptake, clearly indicating that the functional antagonism observed for (±)-**3** is primarily due to the functional antagonism of the (-)-**3** isomer.

Compounds **6**:

Based on the above conformational analysis and the *in vitro* tests described above, the antagonist activity of the most potent compound **6** was tested in a functional assay. Interestingly, although **6** has a much better potency in binding and uptake than **3**, it only has a marginal functional antagonism, significantly less than that of the lead compound **3** (data not shown).

Compounds **19** and **20**:

Out of the new compounds synthesized, analogs (±)-**19** and (±)-**20** were found to have significant functional antagonism. The chiral isomers (-)-**19**, (+)-**20** and (-)-**20** are potent and selective inhibitors at the DAT site, while (+)-**19** has a relatively poor potency (K_i equal to 779 nM). To further investigate the therapeutic potential of these compounds, we evaluated the functional antagonism of (+)-**19**, (-)-**19**, (+)-**20** and (-)-**20**. The results are shown in Table 6.

It was found that in the presence of 10 nM of (-)-**19**, the IC_50 of cocaine in inhibition of DA reuptake was shifted from 297 nM to 529 nM. The value is significantly greater than the theoretical IC_{50} value for cocaine (329 nM) by assuming that cocaine and (-)-**19** have the same binding site to the DAT, indicating that (-)-**19** has a significant functional antagonism at this concentration. Furthermore, a

significant functional antagonism was also obtained for (-)-**19** at 30 nM (Table 6). It is of note that the functional antagonism of (-)-**19** is somewhat better than (-)-**3**. Because (-)-**19** is 3-times more potent than (-)-**3** in inhibition of DA reuptake and has a stronger functional antagonism against cocaine than (-)-**3**, it represents an interesting lead for further investigations for its therapeutic potential for the treatment of cocaine abuse. Functional antagonism testing of (-)-**19** revealed that this isomer also has a significant antagonism against cocaine. Because of its weaker activity, a much higher concentration of (+)-**19** is needed in order to achieve a similar level of antagonism, as compared to (-)-**19**. Therefore, the functional antagonism of (±)-**19** observed at concentrations of 30 and 75 nM is primarily due to the functional antagonism of (-)-**19**. Functional antagonism testing of (+)-**20** and (-)-**20** showed that both stereo-isomers display a significant functional antagonism at concentrations of 10 and 30 nM (Table 5). The functional antagonism of (+)-**20** and (-)-**20** is comparable to that of (-)-3 but is less than (-)-**19**.

Table 6. Functional antagonism against cocaine.

Drugs	IC_{50} (nM) [^{3}H]-Dopamine Uptake (Experimental)	IC_{50} (nM) [^{3}H]-Dopamine Uptake (Theoretical)
Cocaine alone	297 ± 22[a]	
Cocaine + (±)-**3** (50 nM)	470 ± 25	331 ± 11
Cocaine + (±)-**3** (200 nM)	**717 ± 49**	438 ± 22
Cocaine + (-)-**3** (30 nM)	482 ± 8	329 ± 16
Cocaine + (-)-**3** (100 nM)	663 ± 20	430 ± 22
Cocaine + (+)-**3** (300 nM)	464 ± 2	**343 ± 17**
Cocaine + (+)-**3** (1000 nM)	701 ± 58	461 ± 24
Cocaine + (±)-**19** (30 nM)	463 ± 11	373 ± 20
Cocaine + (±)-**19** (75 nM)	595 ± 45	483 ± 23
Cocaine + (-)-**19** (10 nM)	529 ± 61	329 ± 16

Cocaine + (-)-**19** (30 nM)	699 ± 28	405 ± 20
Cocaine + (+)-**19** (200 nM)	563 ± 63	360 ± 18
Cocaine + (+)-**19** (500 nM)	717 ± 110	469 ± 26
Cocaine + (±)-**20** (30 nM)	775 ± 17	363 ± 18
Cocaine + (±)-**20** (100 nM)	945 ± 97	538 ± 29
Cocaine + (-)-**20** (10 nM)	483 ± 12	345 ± 18
Cocaine + (-)-**20** (30 nM)	647 ± 11	479 ± 27
Cocaine + (+)-**20** (10 nM)	451 ± 20	328 ± 13
Cocaine + (+)-**20** (30 nM)	564 ± 32	365 ± 18

[a]Mean ± standard error or range of 2-3 experiments, each conducted using six concentrations of drug in triplicate.

Behavioral Pharmacological Evaluations

Compound **6**:

Although compound **6** only had marginal functional antagonism, in locomotor activity testing, **6** showed partial (40%) locomotor stimulant activity a 100 mg/kg, the maximal dose tolerated in mice, as compared to that of cocaine at the same concentration (Figure 9). Furthermore, at 300 mg/kg, **6** produced a convulsion effect in mice. These data indicate that **6** is capable of entering the brain but seems to behave as a partial agonist. Taken together, the data suggest that **6,** a much more potent DAT inhibitor than cocaine but behaving like a partial agonist, may be a promising lead compound for further evaluation as a potential therapeutic for the treatment of cocaine abuse.

As disclosed *supra*, a focus of the subject invention is to produce by rational methods compounds having significant activity in controlling dopamine flow in the brain, such as by antagonizing cocaine activity *vis a vis* dopamine transporter protein, in dopamine flow control. A potent lead compound (**3**), representing a class of DAT inhibitors, was discovered through 3D-database pharmacophore searching.

Biological evaluation showed that this lead compound has a similar potency to cocaine in binding and uptake but has a significant effect *in vitro* in antagonizing the ability of cocaine to block the reuptake of dopamine. Furthermore, the lead compound has a different selectivity profile from cocaine at the three transporter sites.

Unlike cocaine, the lead compound did not exhibit significant stimulant activity at concentrations from 1 to 30 mg/kg in locomotor activity test in mice. Chemical modifications led to the identification of a much more potent compound **6** with K_i values of 11 nM and 51 nM in binding and uptake, respectively. Importantly, compound **6** is capable of entering the brain but only produces partial locomotor stimulant activity in mice at 100 mg/kg, the maximal dose tolerated.

Molecular modeling studies showed that in addition to the pharmacophore, hydrophobicity and conformational preference are two other important factors for the activities of this class of compounds. Taken together, the data suggest that this class of DAT inhibitors represents promising lead for further development and evaluation as potential therapeutics for the treatment of cocaine abuse.

Compounds **19** and **20**:

Both **19** and **20** are potent and selective DAT inhibitors. Furthermore, (-)-**19**, (+)-**20** and (-)-**20** display significant antagonism. These results suggest that **19** and **20** are potentially interesting candidate compounds for further evaluations. To further assess the therapeutic potential of these new analogs, we have evaluated **19** and **20** in locomotor activity test in mice and drug discrimination test in rats and the results are summarized in Figures 11 and 12. It is of note that due to initial experimental difficulty in obtaining sufficient amount of chiral compounds through HPLC separation for animal studies, these behavioral pharmacological evaluations were performed with the racemates.

Locomotor activity studies in mice showed that both cocaine (3-30 mg/kg, i.p.) and **19** (30-156 mg/kg) produced dose-dependent enhancements in the distance traveled and stereotypic movements in mice (Fig. 11A and 11B). Further increase in the doses of cocaine to 100 mg/kg and of **19** to 300 mg/kg produced convulsions and death. However, cocaine is about 16- (95 % confidence limits: 10-35) and 35-fold (95 % confidence limits: 11-98) more potent (by parallel lines bioassay test) than **19** in increasing the distance traveled and stereotypic movements, respectively. Cocaine (56 mg/kg) is also significantly more efficacious than **19** (156 mg/kg) in increasing the distance traveled ($P < 0.05$) at the maximal tolerated doses. In drug discrimination

testing in rats trained to discriminate 10 mg/kg cocaine from saline, while cocaine (3-10 mg/kg) produced dose-dependent and full substituent, **19** (1-5.6 mg/kg) produced no substituent for cocaine (Fig. 11 C) and **19** did not alter the response rates at the doses tested (Fig 11 D). At 10 mg/kg, **19** disrupted the response rates (data not shown).

Locomotor activity studies showed that **20** (30-156 mg/kg) produced only marginal effect on the distance traveled and lacked stimulant effects on stereotypic movements (Fig. 12A and 12B), much less than cocaine and significantly less than **19**. At a dose of 300 mg/kg, **20** produced convulsions and death in mice. Similar to **19**, **20** (1-5.6 mg/kg) did not generalize to cocaine in drug discrimination testing (Fig. 12C) and did not alter response rates (Fig. 12D) but **20** at 10 mg/kg significantly disrupted responding in rats, suggesting penetration into the brain at this dose.

The primary mechanism underlying the behavioral effects of cocaine is thought to be due to its ability to bind to the DAT and thereby inhibit the reuptake of DA. Both **19** and **20** inhibit the reuptake of DA approximately 3-fold better than cocaine. However, the behavioral data indicate that **19** is less potent and efficacious than cocaine in increasing the locomotor activity and lacked cocaine-like discriminative stimulus effect at non-disruptive doses while **20** produced little or no cocaine-like effects in either locomotor activity or drug discrimination tests. It has been suggested that one promising approach for the development of a cocaine therapy for the treatment of cocaine abuse is to create a compound that potently inhibits DAT and only has some of the behavioral effects of cocaine (a "partial agonist") but is not self-administered. The present data indicate that a potent and DAT selective inhibitor **19** mimics cocaine in increasing locomotor activity but is less efficacious than cocaine. Furthermore, **19** does not generalize to cocaine in drug discrimination test in rats. Moreover, (-)-**19** displays a significant functional antagonism against cocaine in vitro. Taken together, these data indicate that behavioral pharmacological investigations of (-)-**19** may be warranted to fully assess its therapeutic potential for the treatment of cocaine abuse. Efforts to obtain a sufficient amount of (-)-**19** are currently under way and the behavioral pharmacological investigations of this compound will be reported in due course.

EXAMPLES

The following example illustrates the steps of identifying a lead compound, and rationally modifying the lead compound to produce compounds which are useful in the treatment of conditions associated with dopamine transporter protein activity. Data base searching is illustrated based on a pharmacophore obtained through functional analysis of cocaine and some of its known analogs.

Also illustrated are techniques employed in conformational analysis of relevant compounds obtained from the data base. The synthesis of a selected group of compounds from the data base is then illustrated. A method and results obtained for testing some of the compounds in vitro to identify a lead compound (compound 3) is described. Based on rational analysis of the structure of the lead compound, analogs are first designed by modifying certain sites in the lead compound through rational drug design. Synthesis of the designed compounds is then illustrated.

Further illustrated are techniques employed in testing the designed compounds *in vitro*. In vivo testing of a compound having the most adequate activity as shown by the *in vitro* tests (compounds **19** and **20**) are also illustrated.

The techniques, procedures, materials and computer programs employed in the experiments discussed herein are extensively described in the article "Discovery of a novel dopamine transporter inhibitor as a potential cocaine antagonist through 3D-data base pharmacophore searching, structure activity relationships and molecular modeling studies", Wang et al., submitted for publication in the Journal of Medicinal Chemistry. The contents of the article and the references cited therein are hereby incorporated by reference in their entirety.

3D-Database Search

The Chem-X program (version July 96), running on a Silicon Graphics Indigo2 R10000, was used to carry out 3D-database pharmacophore searching. This program has been used to build the NCI-3D database, and was successfully used to carry out 3D database pharmacophore searching. The primary reason for choosing this program was its ability to generate and search multiple conformations for flexible compounds in the database.

The problem of multiple conformations for flexible compounds was found to be of utmost importance in building and searching a 3D-database because flexible compounds may be able to adopt a number of different conformations depending on their environment. It is often difficult to know precisely which conformation is the

biologically active one if a compound can adopt multiple conformations with little energy difference. The biologically active conformations may be different for the same compound when it binds to different receptors. Therefore, it was decided that the best way to handle this situation is to generate and search multiple conformations for flexible compounds. The ability of the Chem-X program to generate and search a large number of conformations for flexible compounds was found to be one key factor for our success in identifying a large number of structurally diverse lead compounds in several projects carried out so far.

We have found that if only single conformations for flexible compounds are searched, many identified lead compounds would be missed. Therefore, multiple conformations for flexible compounds are necessary. However, for a flexible compound with more than 10 single bonds, using a step size of 60 in generating conformations, the total number of possible conformations will exceed 60 million. In practice, we set 3 million conformations as the maximum number to be examined for any single compound.

The current version of the NCI 3D database was built using the July 94 version of the Chem-X program. It consists of 206,876 "open" compounds. Employing the Chem-X program, it is straightforward to search the NCI 3D database of 206,876 "open" compounds for structures that meet the requirements specified in the pharmacophore models. The defined pharamcophore model was built into a pharmacophore query, which included all the specifications as described in the pharmacophore models, such as substructural requirements, and distance and distance ranges between these crucial pharmacophore components. The Chem-X program first checked if the compound has a carbonyl group, an aromatic ring, and a nitrogen attached to at least two carbon atoms and one more carbon or hydrogen. After a compound passes this sub-structural check, it was subjected to a conformational analysis. In this step, conformations were generated and evaluated with regard to geometric requirements specified in the pharamcophore query. Compounds, which have at least one conformation satisfying the geometric requirements, were considered as "hits." "Hits" are only considered as potential candidates for biological testing. A number of additional criteria were used in the selection of compounds for biological evaluation in order to achieve maximum efficiency in the discovery of lead compounds. These criteria include simple chemical structure, small molecule, non-peptidic and chemical structure diversity.

Conformational Analysis

Conformational analysis was performed using the conformational analysis module in the QUANTA program. Generally, if a compound has fewer than five rotatable single bonds, the grid scan conformational search protocol was employed. In this protocol, each rotatable bond was systematically rotated to generate a starting conformation, which was subsequently minimized using the CHARMm program within QUANTA. If a compound has more than five rotatable bonds, a random sampling protocol was used to generate conformations. Up to 5000 conformations were generated and minimized. Energy minimization of each conformation was computed with 5000 iterations or until convergence, defined as an energy gradient of 0.001 kcal mol^{-1} $Å^{-1}$ or less. An adopted basis Newton-Raphson algorithm, implemented in the CHARMm program, was used for energy minimization. A constant dielectric constant (equal to 1) was used throughout all the calculations. Upon the completion of conformation generation and energy minimization, the most stable conformation was identified (the global minimum in vacuum). It is noted, however, that the lowest energy conformation may not be the bioactive conformation, as was shown previously. For this reason, other low energy conformations, typically within 5 kcal/mol of the global minimum were identified. Cluster analysis was performed to determine the number of truly unique conformations (clusters), using the cluster analysis module available in the QUANTA program. These low energy conformational clusters together are likely to include the bioactive conformations for a compound.

Quantitative structure-activity relationships were derived using the genetic approximation algorithm as implemented in the QSAR module of the Cerius molecular modeling package. The partition coefficient between n-octanol and water (logP) values were calculated using the ALOGP method, as implemented in the Cerius molecular modeling package. The molecular volume was calculated using the QSAR module as implemented in the Cerius molecular modeling package.

Synthesis of Lead Compound 3 and its Analogs

General Methods. THF was freshly distilled under nitrogen from sodium benzophenone. ^{1}H and ^{13}C NMR spectra were obtained with a Varian Unity Inova instrument at 300 and 75.46 MHz, respectively. ^{1}H chemical shifts (δ) are reported in ppm downfield from internal TMS. ^{13}C chemical shifts are referenced to $CDCl_3$

(central peak, δ = 77.0 ppm). NMR assignments were made with the help of COSY, DEPT, and HETCOR experiments.

Melting points were determined in Pyrex capillaries with a Thomas-Hoover Unimelt apparatus and are uncorrected. Mass spectra were measured in the El mode at an ionization potential of 70 eV. TLC was performed on Merck silica gel $60F_{254}$ glass plates; column chromatography was performed using Merck silica gel (60-200 mesh). The following abbreviations are used: THF = tetrahydrofuran; DCM = dichloromethane; ether = diethyl ether.

General Procedure for the Synthesis of Compounds 3, 6, and 7.

To an equimolar mixture of aryl methyl ketone and paraformaldehyde in acetonitrile (15 mL/g of ketone), was added methylamine hydrochloride (0.25 eq), and the mixture was refluxed for 20 h in the presence of a catalytic amount of hydrochloric acid (37% w/v, 0.02 mL/lg of ketone). The reaction mixture was cooled to room temperature and, volatiles were removed under reduced pressure. The resulting mass was dissolved in DCM, washed with aq. $NaHCO_3$ solution, water, and brine, dried (Na_2SO_4), and concentrated under reduced pressure. The crude compounds were purified by column chromatography using triethylamine/diethyl ether as eluent to afford the following compounds:

4-hydroxy-l-methyl-4-(4-methylphenyl)-3-piperidyl 4-methylphenyl ketone (3).

White solid; yield 70%; mp 143-145 ^{0}C; ^{1}H NMR (300 MHz, $CDCl_3$) 1.78-1.88 (1H, in), 1.98-2.15 (1H, in), 2.25 (3H, s), 2.40 (6H, s), 2.60-2.85 (3H, in), 2.95 (1H, d, J = 10.5 Hz, 2.9 Hz), 4.20 (1H, dd, J = 11.5 Hz, 3.7 Hz), 5.22 (1H, d, J = 2.5 Hz), 7.07 (2H, d, J = 8.1Hz), 7.25 (2H, d, J = 8.1Hz), 7.40 (2H, d, J = 8.3 Hz), 7.82 (2H, d, J = 8.1Hz); ^{13}C NMR ($CDCl_3$) 20.8, 21.7, 40.2, 45.9, 50.3, 51.5, 54.8, 72.4, 124.4, 128.5, 128.9, 129.5, 133.4, 136.2, 144.3, 144.9, 203.8. Anal. ($C_{21}H_{25}NO_2$) C, H, N.

3, 4-dichlorophenyl 4-(3, 4-dichlorophenyl)-4-hydroxy-l-methyl-3-piperidyl ketone (6).

White solid; yield 71%; mp 120 ^{0}C; ^{1}H NMR (300 MHz, $CDCl_3$) 1.80 (1H, br d, J = 13.9 Hz), 1.95-2.06 (1H, m), 2.42 (3H, s), 2.63-2.72 (2H, m), 2.80 (1H, br d, J = 9.1Hz), 2.90 (1H, dd, J = 11.2 Hz, 8.0 Hz), 4.22 (1H, dd, J = 11.5Hz, 7. 8 Hz), 4.98

(1H, d, J = 2.4 Hz), 7.24 (1H, dd, J = 8.5 Hz, 6.6 Hz), 7.32 (2H, t, J = 8.2 Hz), 7. 5 5 (1H, d, J = 8.3 Hz), 7.64 (1H, d, J = 1.7 Hz), 7.72 (1H, dd, J = 8.3 Hz, 6.5 Hz), 7.96 (1H, d, J = 1.4 Hz); ^{13}C NMR ($CDCl^3$) 39.6, 45.7, 50.5, 50.9, 54.2, 72.1, 123.6, 127.1, 130.0, 130.1, 130.8, 130.9, 132.5, 133.6, 134.9, 138.8, 147.3, 201.2. Anal. ($C_{19}H_{17}C_{14}NO_2$) C, H, N.

2,4-dichlorophenyl 4-(2,4-dichlorophenyl)-4-hydroxy-l-methyl-3-piperidyl ketone (7).

Yellow solid; yield 51%; mp 101-103 ^{0}C; ^{1}H NMR (300 MHz, $CDCl^3$) 1.55 (1H, br d, J = 13.7 Hz), 2.42 (3H, s), 2.68-2.84 (4H, m), 3.00 (1H, dd, J = 11.2Hz, 7. 8 Hz), 4. 5 5 (1H, s), 5.14 (1H, dd, J = 11.5 Hz, 7.6 Hz), 7.00 (1H, d, J = 8.3 Hz), 7.15 (1H, d, J = 1. 8 Hz), 7.18 (1H, d, J = 2.0 Hz), 7.25 (1H, d, J = 2.0 Hz), 7.40 (1H, d, J = 2.0 Hz) 7.89 (1H, d, J = 2.5 Hz); ^{13}C NMR ($CDC1_3$) 34.0, 46.1, 50.8, 51.4, 53.2, 73.1, 127.1, 127.3, 129.9, 130.1, 130.5, 130.8, 132.8, 133.9, 136.3, 138.3, 140.9, 205.7. Anal. ($C_{19}H_{17}C_{14}NO_2$) C, H, N.

General Procedure for the Synthesis of Compounds 8, 9, and 10.

To a solution of the appropriate ketone in THF (10mL/g) was added dropwise ^{1}M DIBAL-H in hexane (2.5 eq) at -78 ^{0}C under nitrogen. After stirring for 2.5 h at the same temperature, the reaction mixture was quenched with aq. NH_4Cl and extracted with DCM, and the organic phase was washed with sat. NaCI solution, dried over Na_2SO_4, and evaporated under reduced pressure. The resulting crude compound was purified by column chromatography using Et_3N/ether as eluent to afford the following compounds:

3-[hydroxy(4-methylphenyl)methyl]-1-methyl-4-(4-methylphenyl)piperdilin-4-ol (8).

White solid; yield 64%; mp 178-180 ^{0}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.60 (1H, br d, J = 14.2 Hz), 1.94-2.06 (1H, m), 2.19-2.43 (11H, m), 2.59-2.71 (3H, m), 4.64(1H, d, J = 4.8 Hz), 6.91 (2H, d, J = 8.0 Hz), 6.96 (2H, d, J = 8.2 Hz), 7.03 (2H, d, J = 8.1 Hz), 7.24 (2H, d, J = 9.3 Hz); ^{13}C NMR ($CDCl_3$) δ 20.9, 21.0, 41.8, 46.2, 50.3, 51.5, 55.1, 73.3, 74.9, 124.6, 126.1, 128.7, 128.8, 135.9, 136.7, 139.7, 144.5. Anal ($C_{21}H_{27}NO_2$) C, H, N.

4-(3,4-dichlorophenyl)-3-[(3,4-dichlorouhenyl)hydroxymethyl]-1-methylpiperidin-4-of (9).

Light yellow solid; yield 79%; mp >220 ^{0}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (1H, br d, J = 13.9 Hz), 1.87-1.98 (1H, m), 2.45 (3H, s), 2.54-2.61 (2H, m), 2.74-2.92 (3H, m), 4.75 (1H, br s), 6.96 (1H, br d, J = 8.5 Hz), 7.05 (1H, s), 7.19-7.28 (4H, m); ^{13}C NMR ($CDCl_3$) δ 39.9, 44.5, 48.0, 48.3, 51.5, 56.0, 73.5, 74.7, 126.3, 126.6, 128.8, 128.9, 130.8, 130.9, 131.5, 131.9, 132.9, 133.1, 144.8, 147.5. Anal ($C_{21}H_{19}C_{14}NO_2$) C, H, N.

4-(2,4-dichlorophenyl)-3-[(2,4-dichlorophenyl)hydroxymethyl]-1-methylpiperidin-4-of (10).

White solid; yield 76%; mp 215-217 ^{0}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (1H, d, J = 7.3 Hz), 2.43 (3H, s), 2.67-2.78 (3H, m), 2.93-3.05 (2H, m), 3.33-3.40 (1H, m), 3.68-3 .73 (1H, m), 6.71 (2H, s), 6.85 (1H, s), 7.29 (2H, d, J = 11.2 Hz), 7.95 (1H, t, J = 8.5 Hz); ^{13}C NMR (CDC_{13}) δ 37.1, 41.9, 46.4, 51.8, 57.8, 75.1, 75.9, 127.0, 128.1, 129.4, 130.0, 131.4, 131.5, 133.0, 133.7, 134.4, 140.3, 144.3. Anal ($C_{19}H_{19}Cl_4NO_2$) C, H, N.

8-aza-1, 5-bis(4-methylphenyl)-8-methyl-2,4-dioxabicyclo[4.4.0]decan-3-one (11).

To a mixture of compound 8 (75 mg, 0.23 mmol) and Et_3N (0.035 mL, 25 mmol) in dry DCM (3 mL) at 0 ^{0}C was added triphosgene (82 mg, 28 mmol). The reaction mixture was stirred for 2 h, during which time it was allowed to return to room temperature, then diluted with DCM (10 mL). The solution was washed with aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The resulting crude compound was purified by column chromatography using Et_3N/ether as eluent to give the title compound as a white solid (35 mg, 44%); mp 195-197 ^{0}C: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.16-2.37 (11H, m), 2.44-2.53 (2H, m), 2.62-2.70 (2H, m), 2.88-2.94 (1H, m), 5.55 (1H, d, J = 4.9 Hz), 6.92 (2H, d, J = 8.1Hz), 6.99 (4H, d, J = 8.6 Hz), 7.12 (2H, d, J = 8.2 Hz); ^{13}C NMR ($CDCl_3$) δ 20.8, 21.0, 30.3, 37.5, 42.8, 45.7, 52.0, 55.2, 81.5, 82.8, 125.0, 125.2, 128.7, 128.9, 134.8, 137.4, 137.6, 137.8, 149.2. Anal ($C_{22}H_{25}NO_3$) C, H, N.

Procedure for the Synthesis of Compound 29.

4-Chloro-3-methylacetophenone (29).

A solution of 4-chloro-3-methyl phenacylchloride (**28**) (4.8 g, 23.6 mmol), sodium iodide (14.2 g, 94.6 mmol) and $SnCl_2$ (14.4 g, 75.7 mmol) in a 5:1 mixture of THF and H_2O was refluxed for 2 h and cooled to room temperature. The organic layer was separated, and the aqueous layer was extracted with ether. The combined organic layers were dried (Na_2SO_4), concentrated, and purified by passing through a small bed of silica gel using ether as eluent to afford the title compound as a colorless liquid (3.55 g, 90%); ^{1}H NMR ($CDCl_3$) δ 2.42 (3H, s), 2.60 (3H, s), 7.42 (1H, d, J = 8.3 Hz), 7.72 (1H, dd, J = 8.3 Hz, 1.7 Hz), 7.83 (1H, s); ^{13}C NMR ($CDCl_3$) δ 20.0, 26.5, 127.0, 129.1, 130.6, 135.4, 136.4, 139.6, 197.1. Anal. (C_9H_9ClO) C, H, N.

General Procedure for the Synthesis of Compounds 12-27.

To an equimolar mixture of aryl methyl ketone and paraformaidehyde in acetonitrile (15 mL/g of ketone), was added amine hydrochloride (0.25 eq), and the mixture was refluxed for 20 h in the presence of a catalytic amount of hydrochloric id (37% w/v, 0.02 mL/lg of ketone). The reaction mixture was cooled to room temperature, and volatiles were removed under reduced pressure. The resulting ass was dissolved in DCM, washed with aq. $NaHCO_3$ solution, water, and brine, dried (Na_2SO_4), and concentrated under reduced pressure. The crude compounds were purified by column chromatography using triethylamine/iethyl ether as eluent to afford the following compounds:

4-Chlorophe yl 4-(4-Chlorophenyl)-4-hydroxy-l-methyl-3-piperidyl Ketone (12).

White olid; yield 88%; mp 168-170 °C; ^{1}H NMR ($CDCl_3$) δ 1.82 (1H, br t, J = 13 .9 Hz), .94-2.07 (1H, m), 2.41 (3 H, s), 2.64-2.73 (2H, m), 2.80 (1H, br d, J = 7.1Hz), 2.9 (1H, dd, J = 11.2 Hz, 2.7 Hz), 4.3 0 (1H, dd, J = 11.5 Hz, 3.7 Hz), 5.10 (1H, d, J 2.7 Hz), 7.22 (2H, d, J = 8.6 Hz), 7.42 (4H, dd, J = 8.8 Hz, 2.9 Hz), 7.82 (2H, d, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$) δ 39.8, 45.2, 50.5, 51.2, 54.5, 72.3, 125.9, 1 8.4, 129.2, 129.6, 132.6, 133.9, 140.8, 145.5, 202.7. Anal. ($C_{19}H_{19}Cl_2NO_2$) C, H, N.

4-Bromophe yl 4-(4-Bromophenyl)-4-hydroxy-l-methyl-3-piperidyl Ketone (13).

Yellow solid; yield 74%; mp 181 °C; ^{1}H NMR ($CDCl_3$) δ 1.80 (1H, br d, J = 13.9 Hz), 1.96-2.08 (1H, m), 2.40 (3H, s), 2.66 (2H, t, J = 11.2 Hz), 2.78 (1H, br d, J = 7.5 Hz), 2.90 (1H, dd, J = 11.3 Hz, 3 .2 Hz), 4.2 8 (1H, dd, J = 11.3 Hz, 3 .4 Hz), 5.08 (1H d, J = 2.2 Hz), 7.29-7.40 (4H, m), 7.5 8 (2H, d, J = 8.3 Hz), 7.75 (2H, d, J =

8.3 Hz); ^{13}C NMR ($CDCl_3$) δ 39.8, 45.9, 50.4, 51.1, 54.4, 72.3, 120.8, 126.3, 129.6, 29.7, 131.4, 132.2, 134.3, 146.0, 202.9. Anal. ($C_{19}H_{19}Br_2NO_2$) C, H, N.

4-Hydroxy-4 (4-iodophenyl)-1-methyl-3-piperidyl 4-iodophenyl Ketone (14).

Light yellow solid; yield 66%; mp 176 ^0C; ^1H NMR ($CDCl_3$) δ 1.80 (1H, br d, J = 14.0 Hz), 1.95-2.03 (1H, m), 2.40 (3 H, s), 2.62-2.71 (2H, m), 2.78 (1H, br d, J = 7.5 Hz), 2 90 (1H, dd, J = 11.0 Hz, 3 .2 Hz), 4.2 6 (1H, dd, J = 11.5 Hz, 3 .4 Hz), 5 .06 (1H, d, J = 2.4 Hz), 7.22 (2H, d, J = 8.3 Hz), 7.5 8 (4H, d, J = 8 .3 Hz), 7.82 (2H, d, J = 8.3 Hz); ^{13}C NMR ($CDCl_3$) δ 39.8, 45.9, 50.3, 51.1, 54.4, 72.4, 92.5, 102.6, 126.6, 129.5, 134.8, 137.3, 138.2, 146.8, 203.2. Anal. ($C_{19}H_{19}Br_2NO_2$) C, H, N.

4-Ethylphenyl 4-(4-Ethylphenyl)-4-hydroxy-l-methyl-3-piperidyl Ketone (15).

Colorless thick syrup; yield 78%; ^1H NMR ($CDCl_3$) δ 1.16 (3H, t, J = 7.6 Hz), 1.25 (3 H, t, J = 7.5 Hz), 1.83 (1H, br d, J = 13 .9 Hz), 2.02-2.11 (1H, m), 2.41 (3H, s), 2.56 (2H, q, J = 15.1Hz, 7.6 Hz), 2.63-2.81 (5H, m), 2.94 (1H, dd, J = 10.8 Hz, 2.5 Hz), 4.40 (1H, dd, J = 11.5 Hz, 3.7 Hz), 5 .23 (1H, d, J = 2.4 Hz), 7.08 (2H, d J = 8.0 Hz), 7.26 (2H, d, J = 8.3 Hz), 7.41 (2H, d, J = 8.3 Hz), 7.85 (2H, d, J = 8.1Hz); ^{13}C NMR ($CDCl_3$) δ 14.9, 15.1, 28.5, 40.0, 45.8, 50.2, 51.4, 54.6, 72.3, 124.4, 127.6, 128.2, 128.5, 133.5, 142.3, 144.4, 150.9, 203.7. Anal. ($C_{23}H_{29}NO_2$) C, H, N.

2-Chlorophenyl 4-(2-Chlorophenyl)-4-hydroxy-l-methyl-3-piperidyl Ketone (16).

White solid; yield 71%; mp 83-85 °C; ^1H NMR ($CDCl_3$) δ 1.58 (1H, br d, J = 13 .7 Hz), 2.42 (3 H, s), 2.66-2.82 (3 H, m), 2.87-2.98 (1H, m), 3.04 (1H, dd, J = 11.0 Hz, 3.4 Hz), 4.60 (1H, d, J = 2.7 Hz), 5.22 (1H, dd, J = 11.5 Hz, 3.9 Hz), 6.94 (1H, d, J = 7.1Hz), 7.07-7.12 (3H, m), 7.25-7.36 (3H, m), 7.92 (1H, d, J = 8.1Hz); ^{13}C NMR ($CDCl_3$) δ 33.9, 46.1, 50.9, 51.4, 53.2, 73.1, 126.5, 127.0, 128.6, 128.8, 128.9, 130.3, 131.3, 131.5, 132.1, 138.2, 142.1, 207.2. Anal. ($C_{19}H_{19}Cl_2NO_2$) C, H, N.

3-Chlorophenyl 4-(3-Chlorophenyl)-4-hydroxy-l-methyl-3-piperidyl Ketone (17).

Light yellow solid; yield 68%; mp 115-117 °C; ^1H NMR ($CDCl_3$) δ 1.80 (1H, br d, J = 11.5 Hz), 1.98-2.09 (1H, m), 2.41 (3H, s), 2.63-2.72 (2H, m), 2.79 (1H, broad d, J = 7.0 Hz), 2.92 (1H, dd, J = 11.2 Hz, 2.9 Hz), 4.3 0 (1H, dd, J = 11.5 Hz,

3.7 Hz), 5.01 (1H, d, J = 2.4 Hz), 7.11-7.20 (2H, m), 7.3 0 (1H, d, J = 7.0 Hz), 7.3 9 (1H, t, J = 8.1Hz), 7.5 3 (2H, d, J = 4.8 Hz), 7.70 (1H, d, J = 7.8 Hz), 7.84 (1H, s); ^{13}C NMR ($CDCl_3$) δ 39.7, 45.8, 50.8, 51.1, 54.3, 72.4, 122.4, 125.2, 126.3, 127.0, 128.3, 129.5, 130.1, 133.9, 134.4, 135.2, 137.1, 149.1, 202.6. Anal. ($C_{19}H_{19}Cl_2NO_2$) C, H, N.

3,4-Difluorophenyl 4-(3,4-Difluorophenyl)-4-hydroxy-l-methyl-3-piperidyl Ketone (18).

White solid; yield 59%; mp 110-112 °C; ^{1}H NMR ($CDCl_3$) δ 1.80 (1H, br d, J = 13.9 Hz), 1.95-2.06 (1H, m), 2.41 (3H, s), 2.66 (2H, t, J = 11.2 Hz), 2.80 (1H, br dd, J = 8.8 Hz, 1.8 Hz), 2.92 (1H, dd, J = 11.2 Hz, 3 .1Hz), 4.23 (1H, dd, J = 11.2 Hz, 3.4 Hz), 5.03 (1H, d, J = 2.5 Hz), 6.99-7.15 (2H, m), 7.21-7.37 (2H, m), 7.68-7.74 (2H, m); ^{13}C NMR ($CDCl_3$) δ 39.7, 45.8, 50.5, 51.1, 54.4, 72.1, 114.1, 114.3, 116.9, 117.2, 117.5, 117.7, 118.0, 120.1, 120.2, 120.3, 125.3, 125.4, 125.5, 125.6, 132.6, 144.2, 147.5, 148.5, 150.6, 152.1, 152.3, 155.9, 201.2. Anal. ($C_{19}H_{17}F_4NO_2$) C, H, N.

3,4-Dimethylphenyl 4-(3,4-Dimethylphenyl)-4-hydroxy-l-methyl-3-piperidyl Ketone (19).

Light yellow solid; yield 76%; mp 90-92 °C; ^{1}H NMR ($CDCl_3$) δ 2.18 (1H, br d, J = 13.9 Hz), 1.99-2.10 (1H, m), 2.16 (3H, s), 2.20 (3H, s), 2.30 (3H, s), 2.31 (3H, s), 2.41 (3H, s), 2.63-2.80 (3H, m), 2.92 (1H, dd, J = 11.0 Hz, 2.8 Hz), 4.38 (1H, dd, J = 11.4 Hz, 3.6 Hz), 5.25 (1H, d, J = 2.7 Hz), 6.99 (1H, d, J = 7.8 Hz), 7.19 (2H, t, J = 7.1Hz), 7.33 (1H, br s), 7.68 (2H, d, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$) δ 19.2, 19.7, 19.9, 20.0, 40.3, 45.9, 50.1, 51.5, 54.7, 72.3, 121.6, 126.1, 126.2, 129.4, 129.5, 129.9, 133.7, 134.8, 136.2, 137.1, 143.7, 144.7, 204.0. Anal. ($C_{23}H_{29}NO$•HCl) C, H, N.

4-Chloro-3-methylphenyl 4-(4-Chloro-3-methylphenyl)-4-hydroxy-l-methyl-3-piperidyl Ketone (20).

White solid; yield 82%; mp 114-116 °C; ^{1}H NMR ($CDCl_3$) δ 1.80 (1H, d, J = 13.9 Hz), 1.99-2.08 (1H, m), 2.30 (3H, s), 2.40 (3H, s), 2.42 (3H, s), 2.64-2.81 (3H, m), 2.92 (1H, dd, J = 11.9 Hz, 3 .4 Hz), 4.32 (1H, dd, J = 11.5 Hz, 3.7 Hz), 5 .10 (1H, d, J = 2.4 Hz), 7.18 (2H, s), 7.40 (2H, t, J = 5.1Hz), 7.66 (1H, dd, J = 8.3 Hz, 2.0 Hz), 7.75 (1H, s); ^{13}C NMR ($CDCl_3$) δ 20.1, 20.2, 39.8, 45.8, 50.3, 51.2, 54.4, 72.2,

123.1, 126.9, 127.4, 128.8, 129.5, 130.6, 132.7, 134.0, 135.7, 136.9, 140.9, 145.5, 203.0. Anal. ($C_{21}H_{23}Cl_2NO2$•HCl) C, H, N.

1-Ethyl-4-hydroxy-4-(4-methylphenyl)-3-piperidyl 4-Methylphenyl Ketone (21).

White solid; yield 60%; mp 115-117 °C; ^{1}H NMR ($CDC1_3$) δ 1.15 (3H, t, *J* = 7.3 Hz), 1.84 (1H, br d, *J* = 13.9 Hz), 1.99-2.10 (1H, m), 2.57 (3H, s), 2.41 (3H, s), 2.53-2.71 (4H, m), 2.90 (1H, broad d, *J* = 11.3 Hz), 3.00 (1H, dd, *J* = 11.2 Hz, 2.6 Hz), 4.36 (1H, dd, *J* = 11.5 Hz, 3.5 Hz), 5.24 (1H, d, *J* = 2.5 Hz), 7.06 (2H, d, *J* = 8.1Hz), 7.24 (2H, d, *J* = 8.1Hz), 7.40 (2H, d, *J* = 8.1Hz), 7.82 (2H, d, *J* = 8.0 Hz); ^{13}C NMR ($CDC1_3$) δ 12.0, 20.7, 21.5, 40.0, 48.6, 50.1, 52.0, 52.5, 72.9, 124.3, 128.4, 128.8, 129.4, 133.3, 136.0, 144.3, 144.8, 203.9. Anal. ($C_{22}H_{27}NO_2$) C, H, N.

4-Chlorophenyl 4-(4-Chlorophenyl)-1-ethyl-4-hydroxy-3-piperidyl Ketone (22).

White solid; yield 91%; mp 151 °C; ^{1}H NMR ($CDC1_3$) δ 1.14 (3H, t, *J* = 7.0 Hz), 1.82 (1H, br d, *J* = 13.9 Hz), 1.95-2.06 (1H, m), 2.5 3-2.71 (4H, m), 2.90 (1H, dd, *J* = 9.5 Hz, 1.7 Hz), 2.98 (1H, dd, *J* = 11.0 Hz, 2.5 Hz), 4.28 (1H, dd, *J* = 11.2 Hz, 3 .4 Hz), 5.10 (1H, d, *J* = 2.4 Hz), 7.22 (2H, d, *J* = 8.5 Hz), 7.42 (4H, dd, *J* = 8.5 Hz, 1.7 Hz), 7.82 (2H, d, *J* = 8.8 Hz); ^{13}C NMR ($CDC1_3$) δ 12.0, 39.7, 48.4, 50.5, 52.0, 52.3, 72.9, 125.9, 128.4, 129.1, 129.6, 132.6, 133.9, 140.7, 145.6, 202.9. Anal. ($C_{20}H_{21}C_{12}NO_2$) C, H, N.

3,4-Dichlorophenyl 4-(3,4-Dichlorophenyl)-1-ethyl-4-hydroxy-3-piperidyl Ketone (23).

White solid; yield 71%; mp 140 °C; 1H NMR ($CDC1_3$) δ 1.11 (3H, t, *J* = 7.3 Hz), 1.78 (1H, br d, *J* = 14.0 Hz), 1.93-2.01 (1H, m), 2.50-2.67 (4H, m), 2.87 (1H, br d, *J* = 11.2 Hz), 2.95 (1H, dd, *J* = 11.0 Hz, 2.7 Hz), 4.19 (1H, dd, *J* = 11.2 Hz, 3.4 Hz), 4.97 (1H, d, *J* = 2.2 Hz), 7.21-7.3 0 (2H, m), 7.50 (1H, d, *J* = 8.3 Hz), 7.61 (1H, d, *J* = 1.9 Hz), 7.69 (1H, dd, *J* = 8.3 Hz, 1.9 Hz), 7.92 (1H, d, *J* = 2.0 Hz); ^{13}C NMR ($CDC1_3$) δ 11.9, 39.5, 48.1, 50.5, 51.9, 52.1, 72.6, 123.6, 127.1, 130.1, 130.2, 130.8, 130.9, 132.5, 133.7, 134.9, 138.9, 147.3, 201.4. Anal. ($C_{20}H_{19}Cl_4NO_2$) C, H, N.

3,4-Dichlorophenyl 4-(3,4-Dichlorophenyl)-4-hydroxy-l-(2-phenylethyl)-3-piperidyl Ketone (24).

Light yellow thick syrup; yield 67%; ^{1}H NMR ($CDCl_3$) δ 1.83 (1H, br d, J = 13.9 Hz), 1.96-2.07 (1H, m), 2.73-3.04 (8H, m), 4.22 (1H, dd, J = 11.2 Hz, 3.7 Hz), 4.98 (1H, d, J = 2.5 Hz), 7.22-7.35 (7H, m), 7.56 (1H, d, J = 8.6 Hz), 7.65 (1H, d, J = 2.2 Hz), 7.72 (1H, dd, J = 8.3 Hz, 2.0 Hz), 7.96 (1H, d, J = 2.2 Hz); ^{13}C NMR ($CDCl_3$) δ 33.6, 39.6, 48.7, 50.6, 52.4, 59.9, 72.7, 123.7, 126.1, 127.1, 127.2, 128.4, 128.6, 130.2, 130.3, 131.0, 132.7, 133.8, 135.0, 139.1, 139.8, 147.3, 201.5. Anal. ($C_{26}H_{23}Cl_4NO_2$•HCl) C, H, N.

4-Bromophenyl 4-(4-Bromophenyl)-4-hydroxy-l-(2-phenylethyl)-3-piperidyl Ketone (25).

Yellow thick syrup; yield 74%; ^{1}H NMR ($CDCl_3$) δ 1.82 (1H, br d, J = 13.9 Hz), 1.97-2.08 (1H, m), 2.74-3.04 (8H, m), 4.28 (1H, dd, J = 11.2 Hz, 3.4 Hz), 5.08 (1H, d, J = 2.4 Hz), 7.21-7.3 8 (9H, m), 7.60 (2H, d, J = 8.6 Hz), 7.74 (2H, d, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$) δ 33.6, 39.7, 48.9, 50.4, 52.5, 60.0, 72.9, 120.8, 126.1, 126.4, 128.4, 128.6, 129.6, 129.7, 131.4, 132.2, 134.3, 139.9, 146.1, 203.0. Anal. ($C_{26}H_{25}Br_2NO_2$) C, H, N.

3,4-Dichlorophenyl 4-(3,4-dichlorophenyl)-4-hydroxy-l-(3-phenylpropyl)-3-piperidyl Ketone (26).

Colorless thick syrup; yield 62%; ^{1}H NMR ($CDCl_3$) δ 1.77-2.02 (4H, m), 2.52 (2H, t, J = 7.8 Hz), 2.61-2.71 (4H, m), 2.86-2.96 (2H, m), 4.18 (1H, dd, J = 11.5 Hz, 3 .7 Hz), 4.96 (1H, d, J = 2.4 Hz), 7.20-7.3 5 (7H, m), 7.56 (1H, d, J = 8.3 Hz), 7.63 (1H, d, J = 1.9 Hz), 7.71 (1H, dd, J = 8.6 Hz, 2.0 Hz), 7.95 (1H, d, J = 1.7 Hz); ^{13}C NMR ($CDCl_3$) δ 28.6, 33.7, 39.6, 48.7, 50.6, 52.5, 57.6, 72.7, 123.7, 125.8, 127.1, 128.3, 130.2, 130.3, 131.0, 131.1, 132.6, 133.8, 135.0, 139.1, 141.8, 147.4, 201.6. Anal. ($C_{27}H_{25}Cl_4NO_2$) C, H, N.

4-Bromophenyl 4-(4-Bromophenyl)-4-hydroxy-l-(3-phenylpropyl)-3-piperidyl Ketone (27).

Yellow thick syrup; yield 69%; ^{1}H NMR ($CDCl_3$) δ 1.77-2.03 (4H, m), 2.52 (2H, t, J = 8.3 Hz), 2.63-2.71 (4H, m), 2.87 (1H, br d, J = 11.0 Hz), 2.95 (1H, dd, J = 11.0 Hz, 2.9 Hz), 4.26 (1H, dd, J = 11.2 Hz, 3.2 Hz), 5.08 (1H, d, J = 2.4 Hz), 7.20-7.41 (9H, m), 7.60 (2H, d, J = 8.3 Hz), 7.74 (2H, d, J = 8.6 Hz); ^{13}C NMR ($CDCl_3$) δ

28.7, 33.7, 39.7, 48.9, 50.4, 52.6, 57.6, 72.9, 120.8, 125.8, 126.3, 128.3, 128.4, 129.6, 129.7, 131.3, 132.2, 134.3, 141.8, 146.2, 203.0. Anal. ($C_{27}H_{27}Br_2NO_2$) C, H, N.

HPLC Separation of Enantiomers

Racemic piperidinols were separated into their individual enantiomers by using a chirex brand HPLC column (Chirex 3018, purchased from Phenomenex, Inc.) which contains (S)-proline covalently bound to γ-aminopropyl silinized silica gel (5 μm particle size) and derivatized via an urea linkage with (R)-1-(α-naphthyl)ethylamine as a chiral stationary phase. The chiral HPLC was performed on a Shimadzu SCL-10A-VP system at a flow rate of 5.0 mL/min at room temperature using hexane/DCM/ethanol-TFA (20-1) as a mobile phase in 87.5 : 10 : 2.5 ratio and UV detection at 254 and 280 nm. The sample for injection was prepared by dissolving racemic piperidinol (5 mg/1 mL) in mobile phase and the separation was earned out by injecting 250 μL on a 250 x 10 mm chiral column. Retention times and rotations of each isomer were given in Table 7.

Table 7. Retention times and rotations of each isomer

Compound #	Isomer	Retention time (t_R) min	Optical rotation	Concentration (%) and solvent
3	(+)-isomer	13.5	+54.0°	0.5, DCM
	(-)-isomer	17.2	-54.0°	0.5, DCM
4	(+)-isomer	14.9	+93.3°	0.4, DCM
	(-)-isomer	18.2	-93.3°	0.4, DCM
19	(+)-isomer	12.1	+44.2°	1.0, DCM
	(-)-isomer	14.$	-44.2°	1.0, DCM
20	(+)-isomer	18.2	+62.3°	0.7, DCM
	(-)-isomer	22.4	-62.3°	0.7, DCM

Pharmacology:

In vitro [^{3}H]Mazindol binding assays

For binding assays, caudate nuclei were homogenized using a polytron in 0.32 M sucrose and centrifuged for 10 min at 1000 x g. The supernatant was resuspended in cold sucrose and centrifuged at 17,500 x g for 20 min. The pellet was resuspended in Krebs-Ringer-HEPES (KRH) buffer consisting of (in mM): NaCl (125), KCl (4.8), MgSO4 (1.2), CaCl2 (1.3), KH2PO4 (1.2), glucose (5.6), nialamide (0.01), and HEPES (25) (pH 7.4) and centrifuged again. Finally, the pellet was resuspended in 30 volumes of buffer, pelleted at 15,000 x g and frozen at -80 °C until used. The striatal homogenates were thawed by resuspension in the buffer described above at 75-125 µg protein/ml and incubated with [^{3}H]mazindol, with or without competing drugs, for 60 15 min in a 4 °C cold room. Non-specific binding was determined with 30 µM cocaine. The bound and free [^{3}H]mazindol were separated by rapid vacuum filtration over Whatman GF/C filters, using a Brandel M24R cell harvester, followed by two washes with 5 ml of cold buffer. Radioactivity on the filters was then extracted by allowing to sit over night with 5 ml of scintillant. The vials were vortexed and counted. IC_{50} values were determined using the computer program LIGAND.

Synaptosomal Uptake of [^{3}H]DA, [^{3}H]5-HT and [^{3}H]NE

The effect of candidate compounds in antagonizing dopamine high-affinity uptake was determined using a method previously employed. For [^{3}H]DA uptake,

dissected rat striata were homogenized with a Teflon-glass pestle in ice-cold 0.32 M sucrose and centrifuged for 10 min at 1000 x g: The supernatant was centrifuged at 17,500 x g for 20 min. This P_2 synaptosomal pellet was resuspended in 30 volumes of ice-cold modified KRH buffer. An aliquot of the synaptosomal suspension was preincubated with the buffer and drug for 30 min at 37 °C, and uptake initiated by the addition of [^{3}H]dopamine (5 nM, final cone). After 5 min, uptake was terminated by adding 5 ml of cold buffer containing glucosamine as a substitute for NaC1 and then finally by rapid vacuum filtration over GF-C glass fiber filters, followed by washing with two 5 ml volumes of ice-cold, sodium-free buffer. Radioactivity retained on the filters was determined by liquid scintillation spectrometry. Specific uptake is defined as that which is sensitive to inhibition by 30 M cocaine. It is identical to that calculated by subtracting the mean of identical tubes incubated at 0 °C. [^{3}H]5-HT and [^{3}H]NE uptake were measured in an entirely analogous fashion using synaptosomes prepared from rat midbrain and parietal/occipital cortex, respectively. Also, specific uptake of [^{3}H]5-HT and [^{3}H]NE were defined in the presence of 10 uM fluoxetine and 1 uM desipramine, respectively.

IC_{50} values were determined by a computer assisted, iterative fit to a four-parameter sigmoidal equation (ALLFIT). 'These values were then converted to K_i values according to the Cheng-Prusoff equation assuming classical competitive inhibition. Preincubation of the drug and synaptosomes at 37° C for 30 min was used to approximate equilibrium conditions as necessary to satisfy the requirements of the Cheng-Prusoff equation.

Functional Antagonism

First, the effects of approximate IC_{10} to IC_{50} concentrations for candidate compounds on the inhibition of [^{3}H]dopamine uptake by cocaine were determined. The IC_{50} value of cocaine in the presence of antagonist was then compared to the IC_{50} value of cocaine alone. Significant differences in IC_{50} values were compared to theoretical IC_{50} values expected from models of "same site" antagonism. IC_{50} values greater than those expected for "same site" antagonism were taken as evidence of functional antagonism. Compounds demonstrating antagonism were tested at lower concentrations to determine their minimum effective concentration. This test was performed under the preincubation conditions described above to allow slowly equilibrating compounds to reach equilibrium. Further, any artifactual differences in

K_i due to differences in temperature, tissue preparation, etc. were negated in this assay as binding of cocaine and the putative antagonists to both the cocaine binding site and the transporter occurred under identical conditions. This insures that a right shift in the cocaine inhibition curve beyond what is expected for two drugs acting at the same site is a true measure of functional antagonism.

Behavioral Pharmacology

Locomoter Activity of Compound 3 through 11

The test compounds were tested for the locomotor effects using male Swiss Webster mice. The potencies and efficacies [not reported] of test compounds to stimulate motor activity were determined and compared with cocaine's effects. The mice were placed in acrylic chambers which in turn were placed inside the activity monitors (Truscan, Coulbourn Instruments, Columbus, Ohio) equipped with infrared light sensitive detectors mounted along two perpendicular walls. Following 1 hr of habituation to test environment, test compounds, saline or cocaine were injected i.p. in a volume of 1 ml/100 g body weight and immediately placed back in the activity monitors. The data was recorded for a minimum of two hours. Each dose was studied in a minimum of ten mice and each mouse was used only once. The dose-effect functions on horizontal distance were constructed after subtracting the saline control group response from the test compound response. The 30-min period responses were computed from the 2 hour data. The 30-min period during which the maximal responses would occur will be used for plotting dose-response function. Data were analyzed using standard analysis of variance and linear regression techniques. ED_{50} values were determined from data using the linear ascending portion of the dose-effect curves.

Locomotor Activity of Compounds 19 and 20

Locomotor activity of male Swiss-Webster mice was recorded using Truscan activity monitors (Coulbourn Instruments, Allentown, PA) and a computer. The activity monitors consisted of acrylic chambers, which were placed inside the sensor ring. The sensor ring was equipped with light-sensitive detectors and the infrared light beams. The X-Y coordinates of the body center of the subject were sampled by scanning the beams and then the successive locations of coordinates were compared. The sum of distances between successive coordinates was measured as the distance

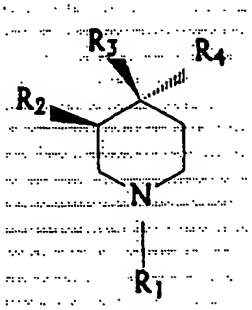
traveled, while the total number of coordinate changes were recorded as the stereotypic movements. Following one hour of habituation to test arenas, several groups of mice were injected intraperitoneally (i.p.) with different doses of cocaine, **19, 20** or its corresponding vehicles, saline and 10% DMSO, in a volume of 10 ml/kg. Locomotor activity was recorded in 10-min bins for the next two hours. The raw data was converted to 30-min totals. The maximal 30-min activity occurred within the 2-hour session following a given test drug injection was determined for each dose level and expressed as the percent of its corresponding vehicle control response for plotting the dose-response curves.

Based on the above results and discussions, 3,4-dichlorophenyl 4-(3,4-dichlorophenyl)-4-hydroxy-l-methyl-3-piperidyl ketone (compound **6**) is presently the most promising candidate of the invention for further development, essentially because of its superior activity and selectivity in binding to dopamine transporter protein.

However, while 3,4-dichlorophenyl 4-(3 ,4-dichlorophenyl)-4-hydroxy-l-methyl-3-piperidyl ketone is currently the most promising candidate for the formulation of therapies based on dopamine flow control, the present invention is broadly directed to the identification of compounds that inhibit dopamine transporter protein.

In a preferred embodiment, such compounds will be synthesized by modification of a lead compound containing a pharmacophore derived from the chemical structure of cocaine, more preferably a pharmacophore containing a secondary or tertiary amine, a carbonyl or an aromatic ring, and most preferably a pharmacophore according to Figure 1.

The design of such compounds is discussed above. In an especially preferred embodiment, these compounds will comprise analogs having the formula (I):



(I)

wherein R_1 is a hydrogen; linear (C_1-C_7) alkyl; branched or cyclic (C_3-C_7) alkyl; halogenated linear, branched or cyclic alkyl; aryl or alkylaryl, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aryl or alkylaryl or connected to the aryl or alkylaryl by a C_1-C_5 alkyl; or an aromatic ring containing one or more hetero atoms selected from N, S, and O, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, and hydroxyl; R_2 and R_4 are independently linear (C_1-C_7) alkyl; branched or cyclic (C_3-C_7) alkyl; halogenated linear, branched or cyclic alkyl; aryl or alkylaryl, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aryl or alkylaryl or connected to the aryl or alkylaryl by a C_1-C_5 alkyl; an aromatic ring containing one or more hetero atoms selected from N, S, and O, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl and an amino group directly linked to the aromatic ring or connected to the aromatic ring by a C_1-C_5 alkyl; C(O)-R', wherein R' is linear (C_1-C_7) alkyl, branched or cyclic (C_3-C_7) alkyl, halogenated linear, branched or cyclic alkyl, aryl or alkylaryl, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aryl or alkylaryl or connected to the aryl or alkylaryl by a C_3-C_5 alkyl, or an aromatic ring containing one or more hetero atoms selected from N, S, and O, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aromatic ring or connected to the aromatic ring by a C_1-C_5 alkyl; primary, secondary or tertiary (C_1-C_7) alcohol, C(O)OR" wherein R" is a linear (C_1-C_7) alkyl, branched or cyclic (C_3-C_7) alkyl, halogenated linear, branched or cyclic alkyl, aryl or alkylaryl, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aryl or alkylaryl or connected to the aryl or alkylaryl by a C_1-C_5 alkyl, or an aromatic ring containing one or more hetero atoms selected from N, S, and O, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aromatic ring or connected to the aromatic ring by a C_3-C_5 alkyl;

C(O)OR''' wherein R''' is a hydrogen, linear (C_1-C_7) alkyl, branched or cyclic (C_3-C_7) alkyl, halogenated linear, branched or cyclic alkyl, aryl or alkylaryl, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aryl or alkylaryl or connected to the aryl or alkylaryl by a C_1-C_5 alkyl, or an aromatic ring containing one or more hetero atoms selected from N, S, and O, optionally substituted with one or more substituents selected from the group consisting of F, C_1, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aromatic ring or connected to the aromatic ring by a C_3-C_5 alkyl; C(O)NH-R'''' or NHC(O)-R'''' wherein R'''' is a hydrogen, linear (C_1-C_7) alkyl, branched or cyclic (C_3-C_7) alkyl, halogenated linear, branched or cyclic alkyl, aryl or alkylaryl, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyls nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aryl or alkylaryl or connected to the aryl or alkylaryl by a C_1-C_5 alkyl, or an aromatic ring containing one or more hetero atoms selected from N, S, and O, optionally substituted with one or more substituents selected from the group consisting of F, Cl, Br, I, linear alkyl, nitro, alkoxyl, hydroxyl, and an amino group directly linked to the aromatic ring or connected to the aromatic ring by a C_1-C_5 alkyl; and R_3 is F, Cl, Br, I, OH, OR''''' or OC=OR''''', wherein R''''' is an alkyl, aryl, aromatic ring containing one or more hetero atoms, or R_3 is a covalent bond replacing the hydrogen in a hydroxyl group of R_2 when R_2 is alcohol or hydroxyl.

Based on the results obtained with the compounds synthesized to date, it is projected that these compounds will have significant therapeutic application. In particular, these compounds should inhibit the binding of cocaine to the DAT and therefore block certain biological effects associated with cocaine. More specifically, these compounds should selectively block the binding of cocaine to the DAT while having a less effect on the binding of dopamine to the DAT.

Thus, the compounds produced according to the invention will be used to treat conditions wherein modulating dopamine reuptake is therapeutically beneficial. This will include conditions that involve cocaine overdose or symptoms associated with cocaine withdrawal during cocaine addiction treatment.

The subject therapies will comprise administration of at least one compound according to the invention in an amount sufficient to elicit a therapeutic response, e.g.,

inhibition of cocaine activity and/or promotion of dopamine reuptake activity in the presence of cocaine.

The compound may be administered by any pharmaceutically acceptable means, by either systemic or local administration. Suitable modes of administration include oral, dermal, e.g., via transdermal patch, inhalation, via infusion, intranasal, rectal, vaginal, topical, and parenteral (e.g., via intraperitoneal, intravenous, intramuscular, subcutaneous, injection).

Typically, oral administration or administration via injection is preferred. The subject compounds may be administered in a single dosage or chronically dependent upon the particular disease, condition of patient, toxicity of compound, and whether this compound is being utilized alone or in combination with other therapies. Chronic or repeated administration will likely be preferred based on other chemotherapies.

The subject compounds will be administered in a pharmaceutically acceptable formulation or composition. Examples of such formulations include injectable solutions, tablets, milk, or suspensions, creams, oil-in-water and water-in-oil emulsions, microcapsules and microvesicles.

These compositions will comprise conventional pharmaceutical excipients and carriers typically used in drug formulations, e.g., water, saline solutions, such as phosphate buffered saline, buffers, and surfactants.

The subject compounds may be free or entrapped in microcapsules, in colloidal drug delivery systems such as liposomes, microemulsions, and macroeinulsions. Suitable materials and methods for preparing pharmaceutical formulations are disclosed in *Remington's Pharmaceutical Chemistry,* 16th Edition, (1980). Also, solid formulations containing the subject compounds, such as tablets, and capsule formulations, may be prepared.

Suitable examples thereof include semipermeable materials of solid hydrophobic polymers containing the subject compound which may be in the form of shaped articles, e.g., films or microcapsules, as well as various other polymers and copolymers known in the art.

The dosage effective amount of compounds according to the invention will vary depending upon factors including the particular compound, toxicity, and inhibitory activity, the condition treated, and whether the compound is administered alone or with other therapies. Typically a dosage effective amount will range from about 0.0001 mg/kg to 1500 mg/kg, more preferably 1 to 1000 mg/kg, more

preferably from about 1 to 150 mg/kg of body weight, and most preferably abou 50 to 100 mg/kg of body weight.

The subjects treated will typically comprise mammals and most preferably will be human subjects, e.g., human cocaine addicts.

The compounds of the invention may be used alone or in combination with other agents. Additionally, the compounds may be utilized with other types of treatments to provide combination therapies which may result in synergistic results.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described.

While the invention has been described in terms of preferred embodiments, the skilled artisan will appreciate that various modifications, substitutions, omissions and changes may be made without departing from the spirit thereof. Accordingly, it s intended that the scope of the present invention be limited solely by the scope of he following claims, including equivalents thereof.